JAN 2

Vol. XVII JANUARY, 1932 No. 4

Medical Lib.

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D., EDITOR
808 Professional Building
Richmond, Va.

ASSOCIATE EDITORS

Pharmacology
DENNIS E. JACKSON, M.D.
University of Cincinnati, Cincinnati

Pathology
PAUL G. WOOLLEY, M.D.
Pasadena

Physiology
J. J. R. MACLEOD, M.B.
Physiological Laboratory, Marischal College, Aberdeen, Scotland

Tuberculosis
GERALD B. WEBB, M.D.
Cragmor Sanatorium, Colorado Springs

Clinical Pathology
W. C. MacCARTY, M.D.
Mayo Clinic, Rochester, Minn.

T. B. MAGATH, M.D.
Mayo Clinic, Rochester, Minn.

Biochemistry
VICTOR C. MYERS, Ph.D.
Western Reserve University, Cleveland

Experimental Medicine
RUSSELL L. HADEN, M.D.
Cleveland Clinic, Cleveland

Immunology
JOHN A. KOLMER, M.D.
University of Pennsylvania, Philadelphia

ROBERT A. KILDUFFE, M.D.
Atlantic City, N. J.

Internal Medicine
GEORGE HERRMANN, M.D.
University of Texas, Galveston

Bacteriology
M. H. SOULE, Sc.D.
University of Michigan, Ann Arbor

Surgery
DEAN LEWIS, M.D.
Johns Hopkins University, Baltimore

PUBLISHED BY THE C. V. MOSBY COMPANY, 3523-25 PINE BLVD., ST. LOUIS.

Entered at the Post Office at St. Louis, Mo., as Second-Class Matter. Additional Entry as Second-Class Matter at Fulton, Mo., Pending.

DR. FREDERICK J. MITCHELL, St. Paul, Minnesota, had been a member of the P.C.A. since February 6, 1908. On October 18, 1931, he was shot and instantly killed by a companion while pheasant hunting. Check for $5,000.00 was promptly mailed to the widow by the P.C.A.

Dr. Paul K. Gaston, Pratt, Kansas, became a member of the P.C.A. in January, 1910, and allowed his policy to lapse at the end of the first quarter. Dr. Gaston and another physician were instantly killed on November 14, 1931, when their car was struck by a passenger train. The total cost to have kept his policy in force until his death would have been $279.00, for which his widow would have received $5.000.00.

In 1931—more than $525,000.00

was paid to Doctors for sick and accident claims of which $90,000.00 was for fatal accidents.

Thirty years' continuous operation under same management

Physicians Casualty Ass'n—Physicians Health Ass'n

OMAHA E. E. ELLIOTT, Secretary-Treasurer NEBRASKA

Over $1,000,000.00 Assets to Protect Contracts

BRING YOUR GLASS PROBLEMS TO

HARRIS COMER

STEELE GLASS COMPANY

145-147 NORTH FOURTH STREET

GLASS APPARATUS
GLASS BLOWING

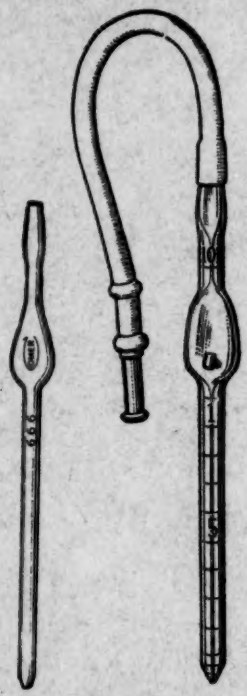
FOR CHEMICAL, RESEARCH, BACTERIOLOGICAL LABORATORIES

We offer the service of skilled workmen and our experience of 40 years in adapting glass to a multitude of scientific and industrial purposes

PHONE LOMBARD 5856

GLASS TUBE GLASS ROD

TRADE COMER MARK



comer

This trade-mark on the back of our Blood Count Pipette means that we guarantee a Greater Accuracy than is required by the U.S. Bureau of Standards.

Illustrating method of breaking tip by using hub as lever

Fig. 1

Patented

Comer's Window Needle *makes it possible to* SEE *that*

- The breakable tip of ampoule is not prematurely broken.
- The Blood shows in Glass Hub when needle is properly introduced into vein.

BLOOD CHEMISTRY KEIDELS

with Sodium Fluoride as used by the Pennsylvania Department of Health under the direction of Dr. John L. Laird

HARRIS COMER — PHILADELPHIA, PA.

The Journal of Laboratory and Clinical Medicine

VOL. XVII ST. LOUIS, MO., JANUARY, 1932 No. 4

CLINICAL AND EXPERIMENTAL

BIOMETRICAL STUDIES OF HEAD LENGTHS OF HUMAN SPERMATOZOA*†

BY G. L. MOENCH, M.D., F.A.C.S., AND HELEN HOLT, B.S., NEW YORK CITY, N. Y.

IN A RECENT article[1] we have dealt with the relationship of sperm morphology to fertility. However, not only the morphology of the spermatozoa and especially of their heads is important, but also the biometrics of these cells. Therefore after the morphology of the semen had been determined, we proceeded to measure the sperm head length.**

Such studies have been made before, but were usually only concerned with the question of sperm dimorphism. Such dimorphism, however, we have found conspicuously absent.[3] On the contrary, the closer the frequency polygon approached a normal frequency distribution, the better in most cases the reproductive fitness of the individual turned out to be, the coefficient of variability especially being that function of the frequency distribution of the population which formed an indicator of the fertility, in the particular case. Thus the fertility decreased as the coefficient of variability increased. No semen specimen, of course, has sperms all of the same size, any more than all cells are ever morphologically perfect. At the same time, we found that the sperm heads from one ejaculate did not vary beyond reasonable limits in normal cases.

Of the total number of 141 different cases in this present series, we were able to calibrate 124. Seven could not be calibrated because too few cells were present, or because the specimen spoiled after our morphologic count was made

*From the Department of Gynecology and the Department of Pathology and Bacteriology of the New York Post-Graduate Medical School and Hospital.

Received for publication, June 2, 1931.

†Work done under a financial grant from the Committee of Maternal Health. Abstract of the second part of the paper presented to the Committee of Maternal Health. An abstract of the first part appeared in the Am. J. Obst. & Gynec. 22: 199, 1931.

**This was accomplished by projecting the image of the stained sperms onto a screen and measuring 300 or more head lengths (at a diameter of 3000 magnifications) with bow dividers.[2]

and before we had time to measure the cells. Ten men of the series had no spermatozoa at all.

Table I* in the last three columns gives the simple function of the graphs obtained in the normal cases. It will be seen that only in a few instances does the standard deviation go above 1.5 and in only four cases is the coefficient of variation above 11, and in only two of these cases (Cases 37 and 41) above 11.5. In Case 41 it took two months for the woman to conceive so that a slight degree of impaired fertility may here be present, although we did not feel justified in excluding the case from the normal list. Case 37 was clinically absolutely normally fertile. The high coefficient here may however be due simply to random sampling, as a few isolated large cells were found in this case. Fig. 1 (Case 92) shows an even more marked example of this. Such isolated, markedly large or small cells may usually be found in any, even the most normal specimen, and while such cells are of course included in tabulating the morphologically abnor-

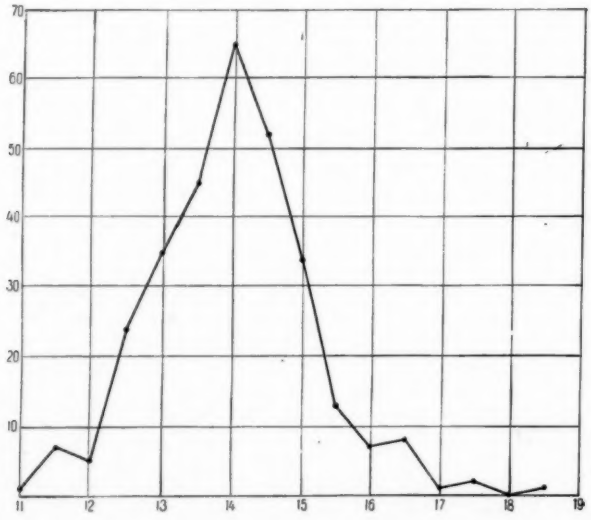


Fig. 1.—Case 92. Graph from a normal case showing several isolated large cells. Such cells do not form a true tail and must be rejected within reason when calculating the functions of the obtained curves.

mal forms, they really should be dropped in calculating the mathematical functions of the graphs, since they acquire a weight entirely out of proportion to their value, as their distance from the mean is squared and thus greatly increases the sum from which the standard deviation (which is one of the factors in determining the coefficient of variability) is calculated. One must, of course, be on his guard in rejecting any data obtained, and if a number of very large or small cells are found repeatedly in a semen specimen, these undoubtedly are significant as indicating a disturbance of spermatogenesis and must never be dropped. Such sperm cells may, in fact, constitute a true tail of the graph, whereas in the other cases we are dealing only with an apparent, or pseudotail.

*Table I may serve as an illustration of the way we tabulated our morphologic and biometrical results in the various groups of patients in this series. Tables for the other groups are omitted to save space. Only the results will be given here.

TABLE I

TABULATIONS OF OBSERVED CELL CHANGES PER 1000 IN 37 CLINICALLY NORMAL CASES, AND THE SIMPLE FUNCTIONS OF THE GRAPHS OBTAINED BY MEASURING 300 SPERM HEAD LENGTHS AT A MAGNIFICATION OF 3000 DIAMETERS, IN THESE SAME CASES.

	CASE	TOTAL ABNORMAL	HEADS ABNORMAL	HEADS ABNORMAL MINUS SIZE CHANGES	M *	P. E.	σ	P. E.	C	P. E.
1	92	361	193	58	12.988	±0.052	1.327	±0.037	10.217	±0.281
2	81	304	111	24	13.447	±0.053	1.368	±0.038	10.173	±0.280
3	45	172	132	27	13.510	±0.044	1.119	±0.031	8.293	±0.228
4	65	223	144	15	13.500	±0.053	1.361	±0.037	10.008	±0.278
5	18	424	133	58	14.672	±0.054	1.383	±0.038	9.426	±0.260
6	40	233	170	68	13.550	±0.047	1.215	±0.033	8.967	±0.247
7	58	150	94	28	13.755	±0.043	1.094	±0.030	7.095	±0.195
8	17	302	151	30	11.810	±0.050	1.283	±0.035	10.864	±0.299
9	83	334	113	27	12.858	±0.046	1.181	±0.033	9.185	±0.253
10	41	233	172	38	12.608	±0.058	1.483	±0.041	11.761	±0.324
11	57	191	142	64	13.547	±0.046	1.187	±0.033	8.762	±0.241
12	48	372	181	31	no calibration—		—too few cells			
13	16	264	183	56	13.765	±0.059	1.526	±0.042	11.086	±0.305
14	27	271	164	73	15.498	±0.057	1.473	±0.041	9.504	±0.262
15	71	362	136	56	13.915	±0.053	1.356	±0.037	9.745	±0.268
16	84	314	112	47	13.468	±0.048	1.242	±0.034	9.219	±0.254
17	50	263	162	64	14.642	±0.065	1.659	±0.046	11.324	±0.312
18	55	too few cells for differential count or calibration—85% of heads normal.								
19	91	234	131	70	14.673	±0.048	1.242	±0.034	8.465	±0.233
20	2	292	162	75	12.518	±0.041	1.052	±0.029	8.403	±0.230
21	28	270	154	54	13.528	±0.056	1.441	±0.040	10.652	±0.293
22	6	201	153	65	13.300	±0.052	1.279	±0.035	9.614	±0.265
23	64	322	151	65	13.337	±0.045	1.150	±0.032	8.623	±0.237
24	37	194	123	23	13.828	±0.063	1.626	±0.045	11.760	±0.324
25	51	263	174	75	13.023	±0.054	1.389	±0.038	10.666	±0.294
26	59	339	122	14	12.462	±0.048	1.220	±0.034	9.790	±0.270
27	46	202	133	30	13.683	±0.051	1.297	±0.036	9.347	±0.261
28	1	213	101	54	14.013	±0.044	1.122	±0.031	8.007	±0.220
29	134	221	142	50	13.225	±0.042	1.072	±0.030	8.106	±0.223
30	52	94	64	14	12.753	±0.039	0.998	±0.027	7.825	±0.215
31	102	176	125	50	14.100	±0.052	1.331	±0.037	9.439	±0.259
32	111	521	123	37	13.137	±0.049	1.262	±0.035	9.606	±0.264
33	112	257	153	70	15.157	±0.058	1.501	±0.041	9.903	±0.273
34	118	142	94	30	13.320	±0.047	1.200	±0.033	9.009	±0.248
35	119	294	151	35	12.507	±0.043	1.117	±0.031	8.923	±0.245
36	125	433	183	55	11.825	±0.043	1.105	±0.030	9.345	±0.257
37	128	372	182	80	13.728	±0.053	1.350	±0.037	9.834	±0.271
					Average		Average		Average	
Breeding record in cases 12 and 18 very good.					13.471	±0.050	1.271	±0.035	9.534	±0.259

* M is the mean, and gives the mean head length in mm. at a magnification of 3000 diameters.
σ is the standard deviation, and is the square of the average deviation of each cell.
C is the coefficient of variability, i.e., the standard deviation multiplied by 100 and divided by the mean.
P. E. is the possible error.

Tables II, III, IV and V of the original article (not reproduced here) show in their averages of the simple functions of the graphs no significant variation of the mean, relatively slight variations in the standard deviation, but most interesting differences in the respective coefficients of variability.

In 11 couples of impaired fertility and clinically abnormal wife the coefficient of variability averaged 10.336 ± 0.287. In 30 couples with impaired fertility but clinically normal woman the average was 12.252 ± 0.337. In 29 sterile couples with clinically abnormal wife the figure is 10.557 ± 0.279, whereas in 34 sterile couples with clinically normal wife the coefficient of variability rose

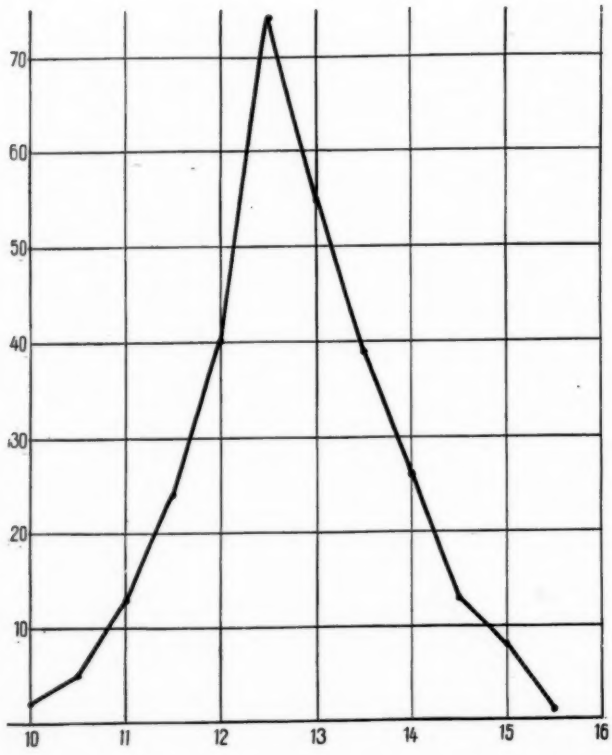


Fig. 2.—Case 52. Graph from a man with a normal breeding record. Coefficient of variation = 7.825 ± 0.215.

to 13.082 ± 0.350. Comparing these results with our normal groups and considering the unavoidable discrepancies which must arise in arranging all our cases only from the woman's side, the results show a very beautiful agreement. In our normal cases and those cases where the woman was abnormal, our coefficient of variability is hardly above 10.5, whereas in those cases where the woman was clinically normal, the figures are above 12 and 13.

The differences present in the graphs from presumably normal and abnormal men are made even more evident by Figs. 2, 3 and 4. Fig. 2 is from a normal man; Fig. 3 from a man whose wife though clinically normal never conceived, and Fig. 4 is from a man who had been married before and had four normal

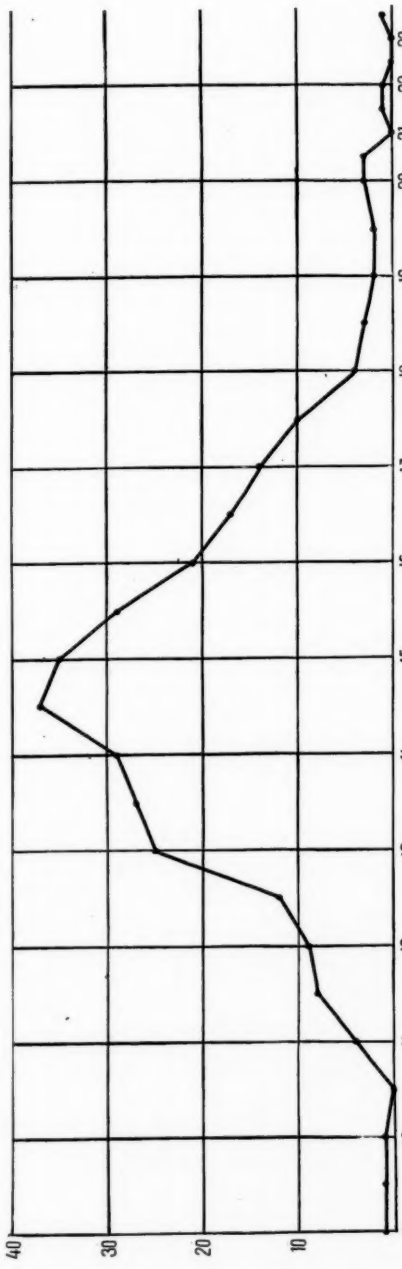


Fig. 3.—Case 23 I. Graph from a man married three years to a normal woman without the wife conceiving, although the couple desires children. Coefficient of variation = 13.809 ± 0.380.

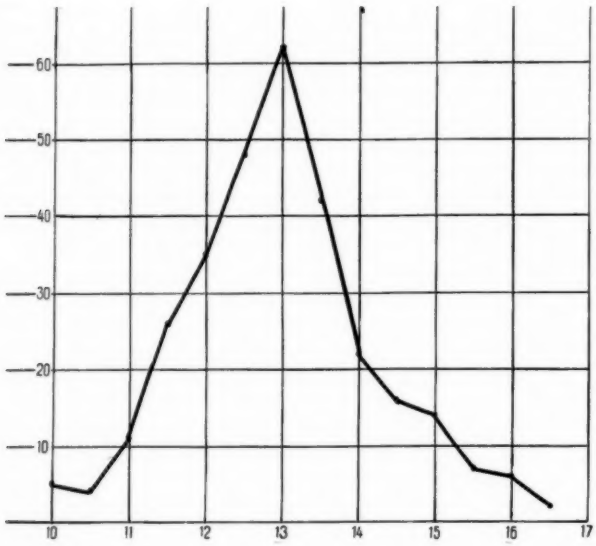


Fig. 4.—Case 105. Graph from a man married six years to a woman with marked ovarian deficiency. From a former marriage this man has four normal children, the youngest seven years old. Coefficient of variation = 9.606 ± 0.265.

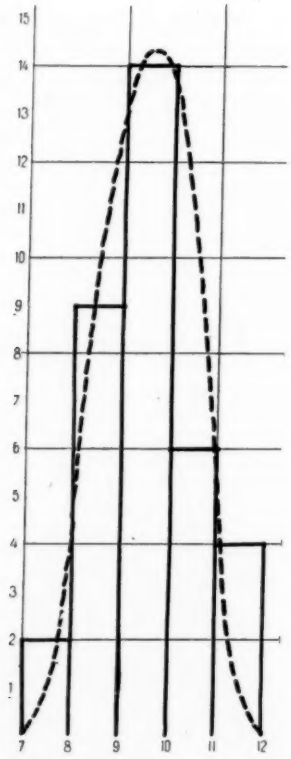


Fig. 5.—Histogram showing the distribution of the coefficients of variation of the sperm head lengths in the 35 normal cases.

children from his first wife, and was now married again six years without the woman having conceived. She was thirty years old, and had a definite ovarian dysfunction.

Since it is impractical to reproduce here more than a few illustrative graphs we have arranged the coefficients of variation of all our cases from the various groups in the form of histograms, which seem to us very instructive. They are

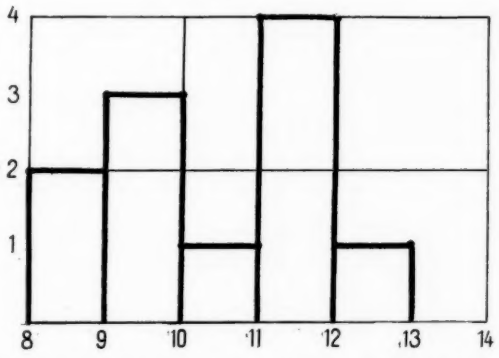


Fig. 6.—Histogram showing the distribution of the coefficients of variation for sperm head length in 11 cases of impaired fertility where the woman was clinically abnormal.

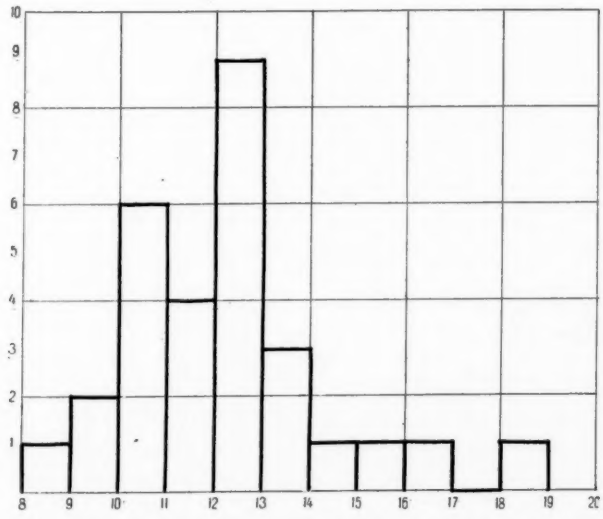


Fig. 7.—Histogram showing the distribution of the coefficients of variation for sperm head length in 29 cases of impaired fertility where the woman was clinically normal.

arranged in class units of one, since this unit is two to four times the usual probable error, and thus mathematically of importance. In Fig. 5 from Table I, we have the 35 normal cases which were calibrated. We see at once that these cases group themselves in a fairly symmetrical almost normal or Gaussian curve with a mean lying between 9 and 10. In Fig. 6 we have too few cases to expect the normal frequency distribution, but we see that here again more cases are below 11 than above it, and only one case above 12. In this last case (Case 104)

the breeding record was difficult to determine, but there is strong evidence that the man was abnormal, as he had been married before and his first wife also had never become pregnant. In some of the cases between 11 and 12 outlying cells were present, besides in some of these the husband, as well as the wife, may have been of impaired fertility. In Fig. 7 we see a decided difference from the two previous histograms. In the first place, although there are almost as many cases as in the normal group, there is no normal frequency distribution, and second, the weight of the graph lies above 12. At the same time, a number of the cases, 13 all told, fall below 12, and even below 11, and therefore within the normal limits. This is easily explained. While it is generally true that the biometrics run more or less parallel to the morphologic tables, this is not necessarily so. A cell may, for instance, be narrow and misshapen, and still be of normal length, and with many such cells present in a specimen, the calibration will give good

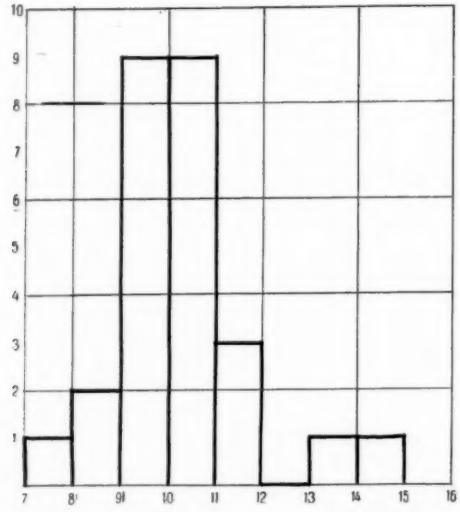


Fig. 8.—Histogram showing the distribution of the coefficients of variation for sperm head length in 26 cases of sterility where the woman was clinically abnormal. Note the relatively normal frequency distribution.

results. The coefficient of variation thus is of value only when it is beyond the normal limits. It might be thought of, therefore, that not only the cell head lengths should be calibrated, but also the width of the sperm head, but the question then arises where such width is to be measured. If we simply take the broadest part of the cell head, the important tapering forms which may be and often are of normal width anteriorly will escape, and if we do not take the broadest part of the cell, it is hard to designate any point at which the measurements are to be made. Carried to the extreme, one would have to make three dimensional measurements of each sperm head, which is of course out of the question. In general, the head length alone will be sufficient, since it must influence, except in rare and unusual cases, the volume of the cell head, and thus the amount of nuclear material present, and in a case where the biometrics really seem to fail, the morphologic examination is always available as an indicator. Thus in 7 of the 13 cases with a fairly low coefficient of variation the cytologic changes were marked. In five of the remaining cases abortions were a dominant feature,

and these may perhaps have been due to some undetected abnormality of the woman. The last case (Case 5) was also one of impaired fertility where sexual overloading may have played a rôle.

In Fig. 8 where the men were presumably normal, we again have a practically normal frequency distribution centering around 9 and 10, with only two cases beyond the normal limits, and as stated above, there is no reason why two abnormal or sterile people may not be married to one another. In Fig. 9 we have instead of the normal frequency distribution, a decided and positive skew, and again, as in Fig. 7, the weight lies on the far side of 12, only seven cases being within the extreme upper limit of the normal. Case 12 (coefficient of variation between 8 and 9) will be taken up separately later. Of the remaining

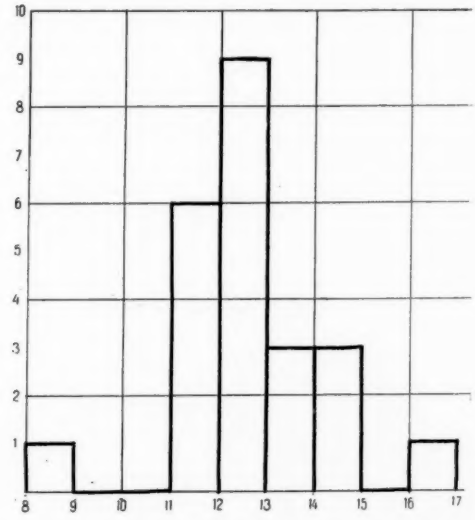


Fig. 9.—Histogram showing the distribution of the coefficients of variation for sperm head length in 23 cases of sterility where the woman was clinically normal. Note the irregular frequency distribution and decided skew to the positive side.

six cases, five showed markedly abnormal cytology, and the last one (Case 22) was apparently unexplained, although the head changes minus the size changes were higher than in the normal cases.

SKEWNESS AND TYPE OF CURVE

Aside from the simple functions of the graphs already given, there are a number of other functions which are significant in biometrics. One of these is skewness, or unequal distribution of the population, producing a lopsided curve, Fig. 10 may serve as an example. Here we have a positive skew, tailing off in the direction of the larger ordinates. We have, therefore, picked out of the 12 normal cases whose graphs appeared most skew, and analyzed these graphs, and have done the same thing with 12 of the abnormal cases, especially such as presented no explanation for the impaired or absent fertility, since both husband and wife appeared normal. Table II gives the complete mathematical analysis of the 12 normal cases and may serve as an example. It is to be noted, however, that the curve types are but rude approximations, since with such a small popu-

TABLE II

MATHEMATICAL ANALYSIS OF GRAPHS FROM TWELVE NORMAL CASES.

CASE	B_1	P. E.	B_2	P. E.	K_2	P. E.	SKEWNESS	P. E.	CURVE TYPE	REMARKS
27	0.116912	0.079438	3.350707	0.369151	0.257546	0.555224	+0.153857	0.048480	IV or VII	
41	0.106451	0.168221	4.452499	3.307564	0.032683	0.068924	—0.096308	0.081774	VII	
52	0.002209	0.001495	3.012722	0.195868	0.088093	0.032034	+0.023354	0.048013	IV	
40	0.000059	0.000075	3.778687	0.213800	0.000029	0.000046	+0.005263	0.050622	VII	
125	0.001061	0.000662	2.865412	0.156928	—0.002924	0.002259	—0.017954	0.047507	VII	
51	0.108978	0.210276	4.929641	5.841000	0.025010	0.120714	+0.087290	0.120325	I or VII	P. E.s only Approximated
37	0.078091	0.055684	3.236743	0.320866	0.249709	1.479720	+0.129769	0.047896	IV or VII	
134	0.018551	0.011487	2.888457	0.167831	—0.050174	0.116820	+0.075223	0.049454	VII	
50	0.008337	0.006659	3.287853	0.311131	0.011483	0.027258	—0.032670	0.049649	VII	
6	0.460093	0.428340	5.084301	4.750680	0.142220	0.412764	+0.200703	0.194700	I or VII	P. E.s only Approximated
92	0.111156	0.128502	4.044401	1.216486	0.049583	0.054127	+0.111255	0.055419	VII	
119	0.036399	0.021456	2.820904	0.157707	—0.059035	0.059968	+0.111363	0.047896	VII	

lation as 300, the probabilities of error frequently become so large that two or three different forms of Pearson's type curves could be fitted to the obtained data, so that we simply had to choose that one which seemed most logical.[4, 5] Due to the carrying of a pseudotail produced by isolated cells in our calculations, some of the graphs approach the heterotypic curves (Case 51, for example).

One thing, however, did stand out from our tables, namely, that the normal cases showed more clearly normal curves (Type 7 of Pearson) than the abnormal cases.

In Table III we present the skewness factors and the relation of this function to its probable error. According to Pearl[6] the value of the skewness factor is unimportant unless it amounts to at least four times its probable error. This we see is not so in any of our normal men, so that these can be considered disposed of immediately. Our abnormal cases, however, show four instances where the skewness factor is more than four times the probable error. In case 101

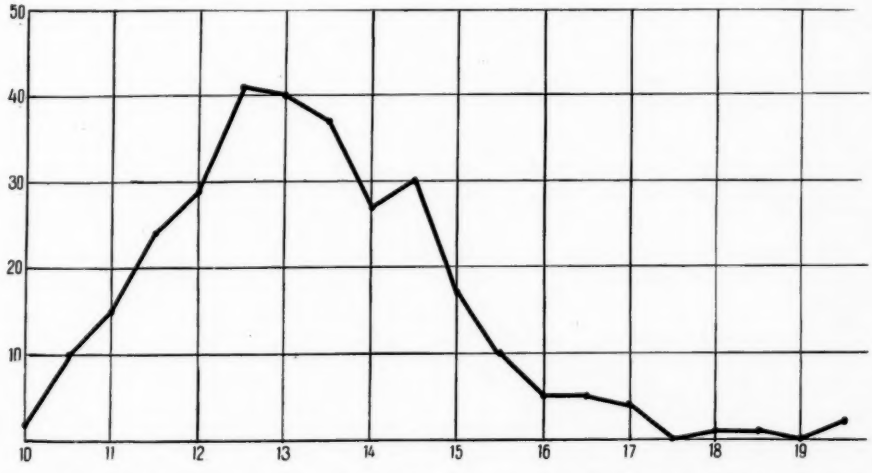


Fig. 10.—Here we have a marked positive skew with a tailing off in the direction of the larger cells. The man had a very poor breeding record.

(Fig. 11) it is so slightly above 4 as hardly to be significant, and in addition, this curve is skew solely because of random sampling. We have on re-calibration found smaller sized cells than the smallest in the present curve, but thought it interesting to show this figure in order to call attention to the possibility of artificially produced abnormalities. The remaining three graphs (not reproduced here), however, showed definite skewness. In two of these, Cases 32 and 109, the cytology of the semen was bad. In one of these two the coefficient of variation was also beyond the normal limits, whereas in the other the only definite abnormality in the biometrics was the skewness. This case (Case 12) is, however, extremely interesting. This was one of our unexplained cases. The wife, married for the first time one and a half years ago, was normal except for a moderate retroversion which we did not deem a sufficient explanation for the existing sterility. The husband had been married nineteen years ago for the first time, and although no contraceptive had ever been used, his wife only had had one

TABLE III

TABLE SHOWING SKEWNESS AND RELATION TO PROBABLE ERROR OF THIS FUNCTION IN EACH CASE ON 12 NORMAL AND 12 ABNORMAL CASES OF TABLES XI AND XII

NORMAL CASES			ABNORMAL CASES		
CASE NO.	SKEWNESS	X. P. E.	CASE NO.	SKEWNESS	X. P. E.
27	+0.153857	3.23	44	—0.000175	0.003
41	—0.096308	1.18	124	+0.014297	0.30
52	+0.023354	0.49	138	+0.069322	1.46
40	+0.005263	0.10	109	+0.545100	6.03
125	—0.017954	0.38	67	—0.108474	1.30
51	+0.087290	0.73	99	+0.598295	3.04
37	+0.129769	2.71	12	+0.297601	5.62
134	+0.075223	1.52	32	+0.293362	4.58
50	—0.032670	0.66	101	+0.192488	4.08
6	+0.200703	1.03	107	+0.380051	3.95
92	+0.111255	2.01	24	—0.127331	2.62
119	+0.111363	2.33	36	—0.118704	2.26

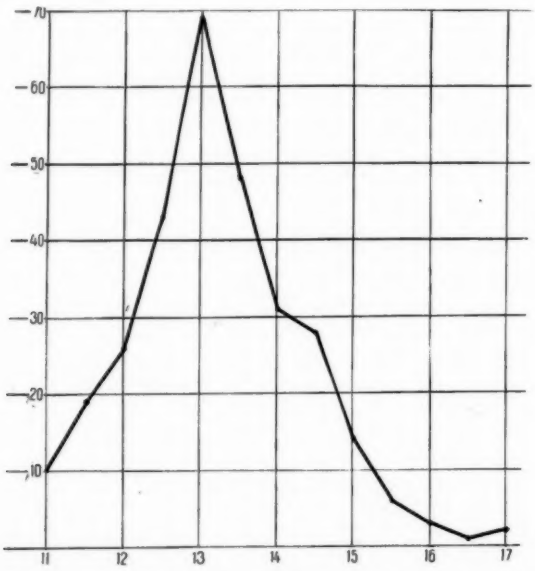


Fig. 11.—Case 101. Graph from a man married to an abnormal woman. Skewness factor only 4.08 times the probable error. Seminal morphology good. Coefficient of variation 8.475 ± 0.237. Skewness due to random sampling, see text.

abortion of two months one year after marriage, and a normal child five years after marriage. In 1915 this man was separated from his first wife and married the present one. The semen examination showed a normal morphology and a (normal) coefficient of variation of only 8.5, so that we were at a loss to explain this case until our complete mathematical analysis revealed the high skewness factor. It may perhaps be doubted that such a delicate factor as skewness, the result solely of advanced mathematical analysis of the frequency distribution polygon, can be significant. At the same time it must be remembered that skewness can only be produced on the negative side by an unusual number of undersized heads, and on the positive side by a great number of abnormally big cells,

and since no significant distortion due to the presence of such sperm heads is found in the semen of normal cases, the condition is abnormal.

CORRELATION OF LABORATORY AND CLINICAL DATA

In investigating the clinical histories of our patients after the semen examination was completed, it was really astounding to see how closely in the great majority of cases, we could predict the clinical breeding record of the case from our microscopic findings, except of course in those cases where we considered the husband normal, but the couple had a bad breeding record due to some pathology of the wife. In thus looking up the clinical history after we had completed our laboratory investigations, we hoped to exclude all subjectivity in the interpretation of our findings. We will, however, not deny that a certain amount of subjectivity may have entered into the interpretation of the clinical histories. At the same time we tried as much as possible to avoid this, and to judge as impartially as we could whether or not the minor lesions which the woman at times presented could possibly explain the abnormalities of fertility found in the couple. We do not at all deny that in thus judging our cases we may not have erred in some. At the same time seminal micropathology and clinical breeding record agreed so closely in the vast majority of our cases that we can hardly consider the possibility that such agreement was the result only of misinterpretation of the clinical records. Besides, such misinterpretation is really possible only in doubtful cases and hardly in those where the couple had normal children in rapid succession, or had none at all.

Because we wanted to avoid the subjective influence as much as possible, all special examinations on the women were also deferred until the semen was examined. Naturally in so doing we at times had disappointments, inasmuch as a man whom we considered of greatly impaired fertility was found to be mated to a woman who had closed tubes. Thus our whole work on this case would become useless, as the man's fertility was not determinable. Such results are, however, to be expected from time to time.

In most cases the morphologic changes and the coefficient of variation ran more or less parallel, but it also occurred that the specimen showed a poor morphologic picture and gave a good biometric result. This has already been explained. On the other hand, due to small, graded size changes, unappreciable under the microscope, under the magnification employed in tabulating sperm morphology, an apparently good cytology may be present, whereas the biometrics show up marked size differences, and result in a high coefficient of variation. A normal cytologic *or* biometric result by itself therefore means nothing, but an abnormal one in either case becomes significant.

In doubtful cases it is of value to count the abnormal sperm heads minus their size changes, as large numbers of narrow, tapering, or misshapen cells are very important. When the figures for this last count go much above 80 or 85 per 1000 the man is at least to be suspected to be of impaired fertility. In Case 76, for instance, the woman was normal, but did not conceive for a number of months, despite normal sex relations. In this case the head changes approached 200 per thousand, the head changes less the size changes were 85 per thousand,

and the biometrics, while well within normal limits, were at least above the average.

It seemed to us that those cases where both the morphology of the semen and the biometrics gave moderately high figures were of lower fertility than those where only one of these two means of examination gave equally high results.

For our studies patients who had been married before were especially desirable. We had, however, only eight such cases (Cases 7, 11, 12, 49, 54, 77, 104, and 105). Of these cases, Case 12 has been discussed under skewness, and Case 11 (sperm motility lost after an attack of influenza) will be taken up later. Case 54 is inconclusive, since both husband and wife were abnormal. In Case 7 the woman had normal children from a previous marriage, and now was apparently sterile. The semen was poor, according to our standards. In Case 49 the woman had been pregnant in a previous and the present marriage, and now had closed tubes, apparently after an abortion. The husband was normal. In Case 77 the woman now married without becoming pregnant, was normal and had been pregnant in a previous marriage. According to our standards the second husband was infertile. Case 104 has already been discussed. Case 105 is shown in Fig. 4 and its legend.

As mentioned in the discussion on biometrics there were among the total number of our patients some in whom we could offer no explanation for the infertility. This is only to be expected. In fact, we would have been extremely suspicious of our methods of investigation had all the cases followed the rule without deviation. Among the 141 married couples examined by us there were 104 (141 total number less the 37 normal) of impaired fertility and sterility. Counting all, even the partially unexplained instances of impaired fertility, we had only eight couples in whom no assignable reason for the disturbed fertility could be discovered. In all of these the wife was apparently normal. It is true that in four of these eight cases we did not examine the woman, but she was reported to be normal. In one of the eight we really had a rather high cell count and biometrics, so that this case may perhaps be partially explained. In two others sexual overloading was present, but the semen showed only changes well within normal limits. One case (Case 20) showed a condition which we believe is not infrequently met with. This woman, after an apparently traumatic first miscarriage, had other spontaneous miscarriages, all about the same time as this one. We can offer no explanation whatsoever for this.

It is we think exceedingly interesting that of our unexplained cases seven were in the group of impaired fertility with abortions as a prominent feature, and only one (Case 22, already referred to) in the sterile group. To us it indicates that the aberrations of the external indicators of normal ovulation (menstruation, vaginal flora, etc.), when of sufficient degree to cause sterility in the female, are generally easily recognizable.

Although thus among our 21 couples in whom spontaneous abortions occurred, there were six (Cases 5, 20, 44, 67, 82, 138) where no explanation could be elicited, including Case 67, with seven, and Case 20 with five spontaneous abortions, it is not without weight that the sperm head abnormalities in this group averaged 200 per thousand, while the coefficient of variation was 11.571 whereas in the intermediate group, where the woman was abnormal, the average

number of sperm head abnormalities per thousand was only 158, and the coefficient of variation but 10.325. This would certainly seem to indicate a participation on the part of the male in the production of these interruptions of gestation. Such participation can naturally be only of the nature of germ plasm defects, and raises the question asked by one of us (M[7]) before, whether the products of gestation in such cases are not inherently abnormal, and abortions only an attempt on the part of Nature to prevent abnormal offspring. Trying to prevent abortions due to these causes thus becomes a procedure of doubtful value, and stresses the fact that before attempting to treat cases of habitual abortion, we must avail ourselves of every possible method to determine the underlying etiology, including a careful examination of the husband. Hydatid mole and other abnormalities of the gestational products considered in this light also take on a different aspect, but unfortunately with the exception of Case 95 (with repeated fetal abnormalities and a semen showing 12 per cent double sperm forms), we had none of the above-mentioned gestational abnormalities in our present series. It is of interest, we believe, to state that Case 95 had a cytology just beyond our assumed normal limits and a normal biometrical status. In no other case, however, did we find any such number of double forms as were present here. Many specimens of course showed double forms, but these never exceeded 1, 2, or at the outside, 3 per cent of the total number of sperms counted.

Of especial interest to us were those cases where apparently the clinical history did not agree with our laboratory findings, and it is worth while to study a few of those cases here.

In Case 11 I obtained the history that the man had been married before and had two normal children, and now was married again without the wife conceiving. As I could not at that time examine the wife, I naturally thought from the history I received that the woman was at fault. The seminal micropathology was however poor, and I found out later that the wife was perfectly normal, and had conceived quickly and easily from three or four different men.

Case 74 is another interesting one. Here the man according to our findings was of impaired fertility, yet the woman claimed she always conceived easily but that contraceptives (coitus interruptus) had been practiced for years. On being advised against this procedure she became "more or less careless," according to her own statements, but so far in six months had not conceived.

Case 75 is an exact duplicate of Case 74, with exactly the same outcome. In addition, the husband had gained eighty pounds since marriage and now was definitely obese. While it is true that Kisch,[8] and also Mettenleiter[9] claim that obesity causes infertility in the female, but not in the male, other authors, as Dickenson and Cary[10] do not agree with this and report cases where the semen improved considerably on reducing the man to normal weight.

In Case 99 we had another instance similar to the last two, but with a very different outcome. Here, too, the woman claimed she conceived easily when contraceptives (coitus interruptus) were not used, but we thought the man to be of a degree of impaired fertility which according to our standards should at least make impregnation difficult. As it happened the woman in the ten days following her next menstrual period for one reason or other had natural intercourse without using any contraceptive and did not become pregnant. She

thereupon became careless but did not conceive until three months later, the pregnancy ending in a spontaneous abortion. The woman was normal except for the fact that she had a moderate retroversion which we did not consider an explanation of this case.

In Case 96 we seemed at first sight to have an instance which was contrary to our theories, since the seminal micropathology was poor, but the woman had had a child only three months before. We learned from the wife, however, that shortly after she became pregnant her husband had fallen off a ladder, had been in the hospital for months, and still was in very poor physical condition.

In Cases 103 and 108 the sperm abnormalities and the biometrics ran to high figures, although both women had a number of young children, but in each case the husband was in poor physical shape.

In Case 137, however, where the woman was three months pregnant when we examined the husband's semen, no history of physical disability of the man could be elicited and still the figures of the seminal examinations were higher than in any other case where the woman had recently been pregnant. However, the breeding record in this case was rather in doubt as the woman had conceived three times, though coitus interruptus was practiced so that it is difficult to say how much of each sterile interval had been due to the contraceptive method employed.

At the same time, it is perfectly possible for a woman married to a man of impaired fertility to happen to conceive on sufficiently often enough repeated intercourse. In another exactly similar case the woman may, however, not happen to become pregnant, and thus in human beings, cases of impaired fertility and lost fertility may offer difficulties of classification as discussed under the heading of the determination of the breeding record.[11] Of course, one can always say that the husband is not the only man living, but I do not believe such an easy explanation to be allowable unless absolute proof of it exists, and there were no indications whatsoever pointing in this direction in this last case. It is of importance here to mention that Williams and Savage[12] have at times found a poor semen in an apparently good breeding bull, but in every such case breeding troubles soon appeared, the disturbance of fertility thus being morphologically discernible in the semen before it became clinically evident.

These eight cases just discussed were about the only ones in our series where at first it appeared as though a poor semen specimen were linked to a clinically good breeding record.

It is striking that in a large proportion of these marriages coitus interruptus was practiced. It has long been recognized even by the laity that coitus interruptus has a deleterious effect, but this was supposed to be mainly on the nervous system. Here, however, we have an indication that this procedure, perhaps due to the frequently concomitant unrelieved congestion, may actually affect spermatogenesis.

We had a total of ten cases (Cases 47, 70, 74, 75, 91, 96, 99, 102, 131, and 137) in whom admittedly coitus interruptus was more or less persistently practiced. In Case 91 this method of contraception had been used for about two years. It was practiced on and off in Case 102; in moderation in Cases 47 and 99, and for a fairly long number of years in Cases 131 and 137, and excessively in Cases 70, 74, and 75.

On looking up the seminal micropathology of these ten patients, we found it normal in Cases 91 and 102, poor in 47, around the limits of the normal in 99, poor in 131, rather poor in 137, and very poor in Cases 70, 74, and 75. While these few cases allow of no conclusions, the findings are at least suggestive and should be followed up. Undoubtedly individual variations will be found here too.

REEXAMINATIONS

Although we made many reexaminations, both on the same and different samples of the same semen, such examinations were mostly for our own information and on semen specimens obtained within a short time of one another. Seven of our abnormal cases, however, returned after three or four months for a reexamination. Their general and sexual habits had been investigated at the time of their first examination and in every case the defects present, especially lack of exercise, etc., resulting in poor general physical health, had been advised about, and rules given to the patient to help him get into better physical trim. Vitamin (especially B, C, and E) containing foods were advised, though generally a deficiency of diet was not to be elicited in these patients. In every case but one (Case 32), where the man admitted not having been able to carry out the instructions very well, the patient at the time of reexamination was both subjectively and objectively in decidedly better physical shape than before, and even Case 32 was somewhat improved.

Table IV shows the results of the various semen examinations made on these seven patients. In every one of these men the semen examination gave figures very much beyond our assumed normal limits when they first presented them-

TABLE IV

REEXAMINATION OF THE SEMEN OF SEVEN MEN OF DECREASED OR ABSENT FERTILITY

CASE NO.	TOTAL ABNORMAL	HEADS ABNORMAL	M	P. E.	σ	P. E.	C	P. E.
10 I	601	273	15.120	±0.075	1.928	±0.053	12.751	±0.351
10 II	503	252	14.767	±0.066	1.684	±0.046	11.404	±0.314
11 I	292	262	12.494	±0.064	1.644	±0.045	13.158	±0.362
11 II	373	231	12.954	±0.059	1.522	±0.042	11.749	±0.324
11 III	314	143	13.398	±0.054	1.397	±0.038	10.427	±0.287
90 I	354	243	13.465	±0.063	1.624	±0.045	12.061	±0.332
90 II	353	251	13.545	±0.056	1.450	±0.040	10.705	±0.398
32 I	514	261	13.615	±0.064	1.632	±0.045	11.987	±0.330
32 II	394	223	13.250	±0.062	1.591	±0.044	12.008	±0.331
23 I	511	364	14.838	±0.080	2.049	±0.056	13.809	±0.380
23 II	423	244	15.150	±0.068	1.742	±0.048	11.495	±0.317
78 I	360	244	15.905	±0.072	1.837	±0.051	11.550	±0.318
78 II	354	271	15.472	±0.061	1.564	±0.043	10.109	±0.278
85 I	413	302	16.020	±0.077	1.984	±0.055	12.385	±0.341
85 II	502	304	15.890	±0.071	1.811	±0.050	11.397	±0.314

selves, so that thus far we have not been able to bring any of them down to normal with the exception of Case 11, and here, unfortunately after an attack of influenza, sperm motility was lost.

Nevertheless, three of the men (Cases 10, 11, and 93) showed improvement both in the morphology and biometrics of the semen, and two others (Cases 90 and 85), an improvement in the biometrical figures, although the morphology remained about the same. In the sixth man the morphology was improved, but the biometrical result about the same as at the first examination. The last patient (Case 79) showed more abnormal sperm heads than before, but a somewhat lower coefficient of variation.

Another fact worth pointing out is that Case 11 had many small sperms which gradually increased decidedly in size with improved physical health, whereas Cases 10 and 78 had large cells which decreased somewhat in size, as shown by the various means. The last change, however, was not so decided as the first, and was approached in value in the opposite direction by Case 23.

SUMMARY

If we summarize here our work on impaired human fertility reported in this and previous articles, including about 60 cases not yet published, we would say:

1. Sterility and fertility are not separate and opposed entities. Fertility is of varying degrees and, starting from the normal, proceeds gradually downward to such low values that clinically sterility is present. Absolute sterility is, however, much less frequent than commonly supposed and being due usually to rather gross lesions, is easily determinable in most instances. In the exact determination of the fertility of any given individual the anamnesis; general, menstrual, sexual, etc., is almost as important as the physical examination. Especial attention must be paid to the practice of coitus interruptus, as it would seem as if this method of contraception detrimentally affects spermatogenesis.

2. In the female the external indicators of normal ovulation (normal ovaries on palpation, normal menstrual cycle, normal relation between the histologic picture of the endometrium and the menstrual periods and a normal [bacillary] flora in a vagina undisturbed for at least four to five days) seem to be sufficiently reliable to allow of the determination of impaired fertility of sufficient degree to cause sterility. Lesser degrees of impaired fertility, resulting in miscarriages and premature labors, seem, however, to be harder to determine and may at times escape detection by the methods thus far employed by us. These cases are, however, being investigated along other lines.

3. Sexual incompatibility from one source or another must at least, judging from the small number of unexplained cases of disturbed fertility in the present series of cases, be considered rare.

4. By means of a careful and exhaustive examination of the semen, it seems possible to determine not only the fertility but also the degree of fertility of any given man. Such a semen examination must include besides other factors a careful consideration of the number of spermatozoa present, their motility, their morphology, and the biometrics of the sperm head lengths. Both the number and the motility of the spermatozoa present in any sample of semen must be judged very guardedly and investigated thoroughly, as purely temporary or

accidental external factors, at times even of a trivial nature, may give rise to misinterpretations.

Judging from our present small series of cases, the morphology of the spermatozoa, especially of the heads, seems to be the best and most reliable indicator of the fertilizing power of these cells. Furthermore the relative number of abnormal heads emitted apparently gives a direct index of the reproductive fitness of the individual. Thus we found no man in our series with more than 19 to 20 per cent abnormal heads who had a good breeding record. In doubtful and borderline cases it is of value also to count the head changes minus the size changes, as narrow and tapering forms are of especially sinister import, and in every case where such a count rose materially above 7.5 to 8.5 per cent disturbed fertility was present.

The biometrical results from measuring the sperm head lengths never gave any evidence of sperm dimorphism. The graphs in the normal cases always approached closely the normal type of curve.

Of the simple functions of the obtained curves, the coefficient of variation is the most important, and was seldom much above 11.0 in a normally fertile man. The upper physiologic limit of this function seems to be around 11.5.

In every case where abnormal curves and coefficients of variation above the normal limits or a mathematically significant skewness was present, the man's breeding record was poor.

In most cases the morphology of the semen and the biometrical results ran parallel. In some instances, however, only the morphology was bad and in others only the biometrics. Thus neither a normal morphology nor a normal curve alone mean normal fertility, whereas an abnormal finding in either signifies a disturbance of spermatogenesis and hence of fertility.

From our figures it would seem as though lesser degrees of disturbed spermatogenesis allow conception to occur, but tend to premature interruption of the pregnancy, but when the fertility sinks as low as six-tenths of the normal value, clinical sterility usually results.

5. The described method of semen examinations is at present only of diagnostic import. It is evident, however, since disturbed spermatogenesis is only a symptom, and careful search must be made in each case for the underlying cause and this treated, that repeated determinations of the cytology and the biometrics of the sperm head lengths will allow of an accurate estimation of the therapeutic results obtained. In the present series of cases we have found spermatogenesis to be most favorably influenced by sexual rest and improvement of the general physical status of the patient.

REFERENCES

1. Moench, G. L., and Holt, H.: Sperm Morphology in Relation to Fertility, Am. J. Obst. & Gynec. **22**: 199, 1931.
2. Moench, G. L.: The Technic of the Detailed Study of Seminal Cytology, Am. J. Obst. & Gynec. **19**: 530, 1930.
3. Moench, G. L., and Holt, H.: Some Observations on Sperm Dimorphism, Biol. Bull. **57**: 267, 1930.
4. Pearson, K.: Tables for Statisticians and Biometricians, ed. 2, London, 1924.
5. Elderton, W. P.: Frequency Curves and Correlation, Layton, London, 1906.
6. Pearl, R.: Medical Biometry and Statistics, Philadelphia and London, 1924, W. B. Saunders Co.

7. Moench, G. L.: A Consideration of Some of the Aspects of Sterility, Am. J. Obst. & Gynec. **13**: 334, 1927.
8. Kisch, E. H.: Sterilitaet des Weibes, Vienna, Urban, 1895.
9. Mettenleiter, M.: Sterilitaet, Munchen. med. Wchnschr. **72**: 977, 1925.
10. Dickinson, R. L., and Cary, W. H.: Esterilidad, J. A. M. A. Span. Ed. **17**: 83, 1927.
11. Moench, G. L.: The Determination of the Breeding Record in Couples with Disturbed Fertility, Am. J. Obst. & Gynec. **19**: 77, 1930.
12. Williams, W. W., and Savage, A.: A Statistical Study of the Head Length Variability of Bovine Spermatozoa and Its Application to the Determination of Fertility, Roy. Soc. Canada, Third Series, **21**: 425, 1927.

THE EFFECTS ON PNEUMOCOCCI OF SODIUM DEHYDROCHOLATE, A BILE SALT DERIVATIVE III*†

By Edwin E. Ziegler, M.D., Washington, D. C.

INVESTIGATION of the relations of the bile salts to pneumococci appears to be promising of practical therapeutic and prophylactic results. The number of papers dealing with this subject is increasing.

This paper represents a continuation of work already published,[1, 2] and deals with the effects of sodium dehydrocholate on tissue, on pneumococci, and on pneumococcus infection.

The chemical nature of sodium dehydrocholate was given in a previous paper.[2] It was first made by Hammarsten[3] in 1881 and was synthesized by Wieland[4] in 1927. The sodium dehydrocholate used in this work was obtained from the manufacturer[5] in a 20 per cent aqueous solution in sterile ampules.

REVIEW OF THE LITERATURE

Rigobello[6] has recently investigated the behavior of the bile acids in the lysis of pneumococci and Downie, Stent and White[7] have recently published an important paper. In this paper it was reported that pneumococci were soluble in all bile salts except sodium dehydrocholate and sodium dehydrodeoxycholate. This is apparently at variance with other work.[2] However, in this latter work the pneumococci used were grown in animals and used without culturing on artificial media. Downie, Stent, and White in their work used twenty-five strains of pneumococci of different types. Sixteen of these were grown for long periods on artificial media. Nine strains were more recently isolated but were nevertheless grown on artificial media. For their tests, cultures in hormone broth were used, 0.9 c.c. of culture being used with 0.1 c.c. of bile salt solution. The sodium dehydrocholate used by Downie, Stent, and White was prepared according to Hammarsten.[3] Considering the great difference in the methods employed, it is not surprising that the results are different. The chemistry of pneumococci grown on artificial media is apparently different from that of those grown in animals.

The English investigators[7] reported a rough Type II pneumococcus resistant to solution in the bile salts. Elton[8] refers to reports that strains of pneumococci have been encountered which were resistant to solution in bile salts. Several hundred strains of pneumococci isolated at the Rockefeller Institute were all reported to be bile soluble.[9]

The work of Downie, Stent, and White is admirable from the standpoint of what takes place in the test tube between various bile salts and relatively avirulent artificially grown pneumococci. From a utilitarian standpoint the toxicity of the bile salts must be given great weight. These workers have shown that

*From The Army Medical School, Department of Bacteriology.
Received for publication, June 10, 1931.
†The work of this paper was performed during a postgraduate course in pathology and bacteriology of the U. S. Veterans Bureau in affiliation with the Army Medical School.

sodium deoxycholate has the greatest pneumolytic power but this salt is also quite toxic. The least toxic of the bile salts, sodium dehydrocholate, does not produce lysis of artificially grown pneumococci as shown by these workers and also by the work of this paper. Nevertheless, it produced lysis of virulent pneumococci taken directly from the animal body in the presence of body fluid.[2]

Barjot[10] has treated persons of the black race suffering from pneumonia, with bile salts intravenously. The preparation which he used was a 7 per cent solution of sodium taurocholate containing 2 per cent of magnesium sulphate. The reason for adding the magnesium sulphate was not given. This worker did not report any local or general toxic effects except a hemoglobinuria when his preparation was injected too rapidly. The color of his patients probably obscured the local injury to the veins. He treated four colored men, three of whom recovered and one died. The one who died had consolidated lungs but cultures for pneumococci were negative. A rabbit injected with blood from this case survived. Barjot points out that four out of seven of his patients treated in other ways died and that the mortality from pneumonia is very high in the colored race, being about 70 per cent.

In the work of Ross[11] where rabbits were fed Pneumocholin (bile salt dissolved pneumococci) mixed with their food, a high degree of immunity was produced. There was also produced considerable immunity with the same method using hydrochloric acid treated pneumococci and macerated organisms. It must be pointed out that these latter two preparations of pneumococci became bile treated when they reached the rabbit's intestine. The simplicity of Ross' method suggests a direct human application. Indeed, the mere swallowing of capsules containing pneumococci or pneumocholin may produce an immunity of practical importance. It may be that the same procedure could also be used in the early treatment of pneumonia to build up an immunity before any natural immunity appears.

EXPERIMENTAL WORK

In the following experiments pneumococci of Type I only were used. They were recently isolated organisms, but were transferred several times on blood agar and in calcium carbonate broth. They were agglutinated by Type I serum. They were soluble in sodium taurocholate but were insoluble in sodium dehydrocholate. The rabbits used were fairly uniform in size and weighed between 2.0 and 2.5 kg.

The sodium dehydrocholate was always administered in a 10 per cent concentration in 0.85 per cent sodium chloride solution; it was prepared by mixing equal volumes of a 20 per cent aqueous solution of sodium dehydrocholate and a 1.7 per cent solution of sodium chloride. In the following experiments where sodium dehydrocholate was used in 10 per cent concentrations in isotonic salt solution, it was prepared in this way. As the drug was obtained in a sterile condition it was only necessary to mix it with sterilized salt solution and to handle it under aseptic conditions.

EXPERIMENT I (TABLE I)

Intraperitoneal Injections of Drug and Organisms.—This experiment was devised in order to obtain an estimation of the degree of the antipneumococcus action of sodium dehydrocholate in vivo. Pneumococci were injected intraperi-

toneally in rabbits and then at different intervals of time were followed by injections of sodium dehydrocholate. In one case the drug was given before the organisms and in another case the organisms and drug were given simultaneously.

All injections were made intraperitoneally. Rabbit 1 was given 1.0 gm. (0.4 of a calculated fatal dose) of sodium dehydrocholate (10 per cent concentration in isotonic salt solution) as a control, and survived without becoming sick. Rabbits 2 and 3, being positive controls, were given 0.5 c.c. and 1.0 c.c. respectively of a 24-hour broth culture of pneumococci. The remainder of the rabbits were given 1 c.c. of the same culture. At varying intervals of time after the injection of the pneumococcus culture, the test rabbits were given one-dose treatments with sodium dehydrocholate. Rabbit 4 received the drug thirty minutes before the organisms. Rabbit 11 received the pneumococci plus the drug in the same syringe. The organisms were thus exposed to the drug less than one minute in this instance.

TABLE I

INTRAPERITONEAL INJECTIONS

ANTIPNEUMOCOCCUS ACTION OF SODIUM DEHYDROCHOLATE

RABBIT	PNEUMOCOCCUS CULTURE	10% SODIUM** DEHYDROCHOLATE	TIME IN MINUTES	RESULT DAYS
1 C	0.0 c.c.	10.0 c.c.	—	S
2 C	0.5	0.0	—	D 1
3 C	1.0	0.0	—	D 1
4	1.0	5.0	-30	D 3
5	1.0	5.0	60	D 3
6	1.0	5.0	150	D 4
7	1.0	10.0	30	D 4
8	1.0	5.0	30	D 4
9	1.0	5.0	5	D 5
11	1.0*	5.0	0	S

D = died; S = survived.
*Drug and organisms given together.
**In isotonic salt solution.

Although the pneumococci were not dissolved on being exposed to sodium dehydrocholate, they did not grow on blood agar and they did not kill Rabbit 11, nor make him sick. The sooner the sodium dehydrocholate was administered after the pneumococci the longer the test animals lived.

EXPERIMENT I-A

Effect of Sodium Dehydrocholate on Veins and Peritoneum.—The following experiments were performed to determine the effect of aqueous and of isotonic solutions of sodium dehydrocholate on veins and peritoneum.

Rabbits 10 and 12 were given injections intravenously of sodium dehydrocholate in 20 per cent aqueous solution. This solution could be injected only with great difficulty as it caused the animals discomfort with consequent struggling.

These rabbits were also given intravenous injections of sodium dehydrocholate 10 per cent in 0.85 per cent sodium chloride solution. This mixture could be given as readily as isotonic salt solution alone with no discomfort to the animals.

Rabbits 10 and 12 were each given intraperitoneally 0.5 gm. of sodium dehydrocholate 10 per cent in 0.85 per cent sodium chloride solution. Autopsy of these animals after several days revealed no evidence of peritoneal injury. There was a slightly increased amount of peritoneal fluid, but no adhesions, fibrinous exudate or other evidence of peritoneal injury.

EXPERIMENT II (TABLE II)

Intravenous Injections of Drug and Organisms.—Since the results of Experiment I were encouraging a similar experiment was undertaken making all injections in the rabbits' ear veins. The sodium dehydrocholate was given in a 10 per cent concentration in isotonic salt solution. A 24-hour broth culture of pneumococci was used. In this experiment four daily injections of the drug were given while only one dose was given in Experiment I. The first dose of the sodium dehydrocholate was given on the same day that the culture of pneumococci was given. The time interval is given in Table II. The controls died in approximately one day. Rabbit 16 died in one day; its back was accidentally broken. One rabbit survived; one lived approximately four times as long as the controls and another six times as long.

Rabbit 1, used as a control for the drug in Experiment I, was again used in this experiment. Ten minutes after receiving a fatal injection of pneumococci it was given 1 c.c. of 10 per cent sodium dehydrocholate in 0.85 per cent sodium chloride solution and 0.5 c.c. of pneumococcus culture in the same syringe. No further treatment was given. This rabbit survived.

Rabbit 11, a survivor from Experiment I, was included in this experiment and was given a fatal dose of pneumococci but no drug. It was injected on Feb. 19, 1931, intraperitoneally with a mixture of pneumococcus culture and 10 per cent sodium dehydrocholate. On Mar. 10, 1931, nineteen days later, it received in Experiment II a fatal injection of pneumococci but survived without becoming sick.

TABLE II

INTRAVENOUS INJECTIONS

ANTI-PNEUMOCOCCUS ACTION OF SODIUM DEHYDROCHOLATE

RABBIT	3 10–31 PNEUMOCOCCI DOSE BROTH	SODIUM DEHYDROCHOLATE 10 PER CENT**					RESULT
		INTERVAL	3–10–31	3–11–31	3–12–31	3–13–31	
14 C	0.2 c.c.	—	0	0	0	0	D 32 hr.
15 C	0.4	—	0	0	0	0	D 20 hr.
16	0.4	10 min.	2. c.c.	0	0	0	D Acc.
17	0.4	15 min.	1. c.c.	2. c.c.	1. c.c.	1. c.c.	S
18	0.4	3 min.	1. c.c.	2. c.c.	1. c.c.	1. c.c.	D 4
19	0.4	10 min.	2. c.c.	2. c.c.	1. c.c.	1. c.c.	D 6
1	0.4	10 min.	*1.5 c.c.	—	—	—	S
11	0.4	—	—	—	—	—	S

*1.0 c.c. 10 per cent sodium dehydrocholate + 0.5 c.c. pneumococcus culture.
D = died; S = survived. C = control. Acc. = accidentally.
**In isotonic salt solution.

On autopsy of Rabbit 18, there was recovered from the heart's blood a growth of B. coli but no pneumococci. Pneumococci were recovered from Rabbit 19 in pure culture.

EXPERIMENT II-A

Subcutaneous Injection of Sodium Dehydrocholate.—In order to determine the effect of subcutaneous injection of sodium dehydrocholate, a 10 per cent concentration of this drug in 0.85 per cent NaCl solution was used. Rabbits 1, 11, 17, 20, and two guinea pigs were injected subcutaneously, after clipping the hair, with amounts varying from 1 to 5 c.c. After twenty-four hours there was no evidence of any tissue damage.

EXPERIMENT III (TABLE III)

Immunity Tests.—The results of Experiments I and II indicated that there was some immunity produced against pneumococci by a mixture of these organisms and sodium dehydrocholate. In this series, Rabbits 1, 11, and 17 received immunizing injections as shown in Experiments I and II and survived. They were used again in this experiment to see if their immunity continued.

Rabbits 24, 25, 27, and 30 received injections of an antigen prepared by mixing equal amounts of an aqueous solution of 20 per cent sodium dehydrocholate and a 24-hour pneumococcus broth culture just before using. It is proposed

TABLE III

INCREASED RESISTANCE TO PNEUMOCOCCI DUE TO PNEUMOCHOLIN-D

RABBIT	PNEUMOCHOLIN-D 3-20-31	4-14-31 24-HR. BROTH CULTURE PNEUMOCOCCI	RESULT DAYS
281 C*	0	0.2 c.c.	D 2
20 C	0	0.2	D 1
1	Exp. II	0.2	D 7
11	Exp. I	0.2	S
17	Exp. II	0.2	S
24	4 c.c. Int.	0.2	S
25	3 c.c. Subc.	0.2	D 4
27	3 c.c. Int.	0.2	D 8
30	2 c.c. Int.	0.2	D 7

*C = control; D = died; S = survived. Pneumocholin-D = equal parts 20 per cent sodium dehydrocholate in aqueous solution and 24-hr. pneumococcus broth culture. Int. = intravenously; Subc. = subcutaneously.

to call this antigen "Pneumocholin-D" as cholic acid is characteristic of all the bile salts. "D" represents the dehydrocholate treated pneumococci, "T" the taurocholate product, "G" the glycocholate pneumococcus lysate, etc. This experiment indicates that Pneumocholin-D increases the rabbit's resistance. Only one dose of the Pneumocholin-D was given. It remains to be seen whether repeated doses will further increase the resistance.

EXPERIMENT IV

Noneffectiveness of a Berkefeld Filtrate.—Pneumococci were grown on blood agar. A heavy suspension of these organisms was made with isotonic salt solution. Then equal parts of the suspension and 20 per cent sodium dehydrocholate

in aqueous solution were mixed and left to stand for three days at room temperature. Lysis was only partial. The mixture was sterile when cultured on blood agar. The mixture was then filtered through a Berkefeld-N filter. The filtrate was divided into two fractions one of which was heated to 60°C. for one hour. The two fractions were then injected subcutaneously in rabbits. The unheated fraction was injected in Rabbits 21C, 22, 23, 26, 28, and 29. The heated fraction was injected in Rabbits 274C, 275, 276, 277, 278, and 279. Neither fraction produced any evidence of immunity when the rabbits were given fatal doses of pneumococci.

EXPERIMENT V

Effect of Sodium Taurocholate and Glycocholate on Tissue.—The work of this experiment was done with that of a former paper[1] but was unpublished. A 10 per cent concentration of a mixture of sodium taurocholate and sodium glycocholate in 0.85 per cent sodium chloride solution was injected subcutaneously on the abdomen of several rabbits. The injection caused a disagreeable sensation and it was sometimes necessary to make several injections to give the amount of drug desired. These injections caused a local tissue necrosis with eventual sloughing. The inner surfaces of the sloughs when removed were sterile. Local damage to the veins of man by these salts has already been reported.[1] Similar damage and a painful sensation was caused by injecting these salts in the ear veins of rabbits.

DISCUSSION

In a previous paper[1] the theory was advanced that bile salts were used by the body in overcoming pneumococcus pneumonia. This theory was based on the work of Elton[8] who demonstrated that the icterus index was increased (within the latent zone) in cases of lobar pneumonia. The identification of the bile salts as such in the blood, has not heretofore been possible. There seems to be no reliable chemical test for the bile salts. The Pettenkofer test formerly relied upon is not specific. A new method for the determination of bile salts has recently been advanced by Duco and Panza.[12] The work of Elton on the blood of pneumonia patients should be repeated using a specific test for the bile salts.

In the chemotherapy of such a disease as lobar pneumonia, the question arises as to whether the injected drug reaches the diseased lung. It is believed that it would. Although in a consolidated lung there may be blocking of some arterioles or capillaries, such blocking could not be extensive. Closing of the vessels would result in infarction and certainly this does not occur, at least in those cases which recover. Resolution could not take place if the blood supply to a consolidated area of lung were closed off. Consolidation is produced by exudation into the bronchial system and alveoli and it is probable that the blood supply is never seriously impaired by the consolidation alone. The lung is an extremely vascular organ having a double blood supply.

The nature of the phenomenon of the solution of pneumococci by bile salts is not definitely determined.

The theories to be considered are (1) chemical reaction, (2) catalysis of normal autolysis, and (3) low surface tension.

A direct chemical reaction between the bile salts and some substance or substances in the pneumococcus is the most probable explanation.

Goebel and Avery[13] in 1929 showed that the solution of pneumococci by bile salts is not always accompanied by the same proteolysis that takes place in normal autolysis and that the bile salts act independently of autolytic enzyme. Bile salt solution of pneumococci occurs most rapidly at 37°C. and is retarded both by lower and higher temperatures, in the same manner as autolysis.

Low surface tension is not responsible for the lysis of pneumococci, as shown by workers already mentioned.[7]

While the explanation of this phenomenon, the solubility of pneumococci in bile salts, is not at present definitely known it should not deter the performance of therapeutic investigations with the bile salts.

There seems to be a certain amount of irregularity in the solubility of different strains of pneumococci. Reimann[14] showed that by the repeated growth of pneumococci in plain broth, to which was added increasing amounts of bile, the organisms became acclimatized to as high as 75 per cent of ox bile. Reimann demonstrated that these organisms after growth in animals were again bile soluble. Also that insolubility in bile was associated with loss of virulence.[15]

The study of the amino-cholic acids, characteristic of bile, and their derivatives, is opening up a vast field of biologic relationships which needs extensive study. The work of this paper creates more problems than it solves so that definite conclusions are deferred pending further investigation.

SUMMARY

1. Pneumococci of Type I, grown on artificial media, were not dissolved by sodium dehydrocholate in this work but were killed or rendered avirulent so that they would not grow on artificial media nor produce infection.

2. Sodium dehydrocholate has an antipneumococcus action in the animal body.

3. A mixture of sodium dehydrocholate and of pneumococci, when injected into rabbits, produces a degree of immunity to pneumococcus infection.

4. Sodium dehydrocholate does not produce local tissue injury as does sodium taurocholate and glycocholate.

The author wishes to express his appreciation to Col. E. B. Vedder and Maj. J. S. Simmons, for permitting this work to be done with facilities at the Army Medical School.

REFERENCES

1. Ziegler, E. E.: The Specific Effect of Bile Salts on Pneumococci and on Pneumococcus Pneumonia, Arch. Int. Med. **46**: 644, 1930.
2. Ziegler, E. E.: Sodium Dehydrocholate, Its Specific Effect on Pneumococci, II, J. Lab. & Clin. Med. **16**: 868, 1931.
3. Hammarsten, O.: Dehydrocholic Acid, A New Oxidation Product of Cholic Acid, Ber. d. deutsch. chem. Gesellsch. **14**: 71, 1881.
4. Wieland, Heinrich: The Synthesis of the Bile Acids, XXVII, Ztschr. f. physiol. Chem. **167**: 70, 1927.
5. Riedel de-Haen, Inc., 105 Hudson St., New York City.
6. Rigobello, G.: Behavior of the Bile Acids in the Lysis of the Pneumococcus, Soc. internaz. di microbiol., Boll. d. sez. ital. **2**: 330, 1930.
7. Downie, A. W., Stent, L., and White, S. M.: The Bile Solubility of Pneumococcus, With Special Reference to the Chemical Structure of Various Bile Salts, Brit. J. Exper. Path. **12**: 1, 1931.

8. Elton, N. W.: Latent Jaundice of Lobar Pneumonia, J. Michigan M. Soc. **28**: 451, 1929; Icterus Index Studies in Lobar Pneumonia, II, New England J. Med. **201**: 611, 1929; Serum Pigmentation and Kinetics of Latent Jaundice of Lobar Pneumonia, J. Detroit Coll. Med. & Surg. **1**: 34, 1930.
9. Avery, Chickering, Cole, Dochez: Monograph No. 7, p. 14, Rockefeller Institute for Medical Research, Oct. 16, 1917.
10. Barjot: The Treatment of Pneumococcus Septicemia by the Intravenous Injection of Bile Salts, Bull. Acad. de med., Paris **100**: 898, 1928.
11. Ross, V.: Oral Immunization Against the Pneumococcus, Use of Bile Salt Dissolved Organisms, etc. Time of Appearance of Immunity and Dosage, J. Exper. Med. **51**: 585, 1930.
12. Duco, C. L., and Panza, P. T.: New Method of Determination of Bile Acids in Body Fluids; Physiologic Cholaluria, Semana med. **1**: 1193, 1930.
13. Gobel, W. G., and Avery, O. T.: J. Exper. Med. **49**: 267, 1929.
14. Reimann, H. A.: J. Exper. Med. **41**: 587, 1925.
15. Reimann, H. A.: Ibid. **45**: 807, 1927.

THE DETOXIFICATION OF COCAINE, PICROTOXIN AND STRYCHNINE BY SODIUM AMYTAL*

By Edward E. Swanson, Indianapolis, Ind.

KNOEFEL, Herwick and Loevenhart[1] have shown that sodium amytal reduces the toxicity of cocaine, procaine and butyn. Clinically, Zerfas and McCallum[2] have used the drug for cocaine and strychnine poisoning. The purposes of this investigation are to ascertain definitely in experimental animals: (a) Whether or not sodium amytal detoxifies the convulsant drugs which act on the parts of the central nervous system other than the cerebrum; (b) whether or not sodium amytal affords greater protection against poisoning by intravenous than by oral administration; and (c) the efficiency of sodium amytal in preventing the occurrence of convulsions induced by these drugs. Picrotoxin and strychnine were selected for investigation since the former is known to cause convulsions chiefly by the stimulation of the medulla, and the latter by that of the spinal cord. Cocaine which is known to produce convulsions by its action on the cerebrum was also studied under the same conditions for comparison.

Rabbits were used in all experiments. The procedure was the same as employed by Knoefel, Herwick and Loevenhart,[1] that is, the poison is injected subcutaneously, and sodium amytal is given simultaneously by vein or by mouth.

As shown in Tables I, II and III, sodium amytal uniformly detoxifies each of the three poisons. Our figures for cocaine with sodium amytal given by vein completely agree with those reported by Knoefel, Herwick and Loevenhart.[1] With sodium amytal given orally the M.L.D. of cocaine is 175 mg. per kg. as against 400 mg. per kg. of cocaine when sodium amytal was given by vein. For picrotoxin alone the M.L.D. is 2.5 mg. per kg. as shown in Table II. With sodium amytal (intravenously) the M.L.D. of picrotoxin is 8 mg. per kg. and with sodium amytal (orally) the M.L.D. is 6 mg. per kg. As shown in Table III the M.L.D. of strychnine alone is 0.6 mg. per kg. With sodium amytal by vein the M.L.D. of strychnine is 4 mg. per kg. and with sodium amytal by mouth the M.L.D. is 2.4 mg. per kg. The detoxifying value of sodium amytal is greater when it is administered intravenously than orally. The difference between the two routes of administration is striking with cocaine and strychnine, but relatively less so with picrotoxin.

There is an interesting relationship in the subcutaneous and intramuscular injection of sodium amytal. With sodium amytal injected subcutaneously (60 mg. per kg.) and intramuscularly (150 mg. per kg.), the M.L.D. of cocaine is the same as with cocaine alone. With picrotoxin and strychnine the prophylactic action of sodium amytal (subcutaneously and intramuscularly) is even greater than by vein or mouth. The M.L.D. of picrotoxin is 15 mg. per kg. with sodium amytal subcutaneously and 10 mg. per kg. with sodium amytal intramuscularly.

*From the Lilly Research Laboratories.
Received for publication, June 22, 1931.

TABLE I

DETOXIFICATION OF COCAINE HYDROCHLORIDE BY SODIUM AMYTAL

WITHOUT SODIUM AMYTAL					WITH SODIUM AMYTAL 10%, 50 MG. PER KG., INJECTED INTRAVENOUSLY					WITH SODIUM AMYTAL, 10%, 150 MG. PER KG., ADMINISTERED ORALLY				
NO. OF ANIMALS	DOSE OF COCAINE IN MG. PER KG.	NO. DIED	NO. SURVIVED	M.L.D. IN MG. PER KG.	NO. OF ANIMALS	DOSE OF COCAINE IN MG. PER KG.	NO. DIED	NO. SURVIVED	M.L.D. OF COCAINE IN MG. PER KG.	NO. OF ANIMALS	DOSE OF COCAINE HCl IN MG. PER KG.	NO. DIED	NO. SURVIVED	M. L. D. OF COCAINE
5	25	0	5		5	200	0	5		1	100	0	1	
5	50	0	5		5	250	0	5		1	125	0	1	
5	75	1	4		5	300	1	4		5	150	2	3	
5	100	4	1	100	5	350	1	4		3	175	3	0	175
					5	400	4	1	400					

TABLE II

DETOXIFICATION OF PICROTOXIN BY SODIUM AMYTAL

WITHOUT SODIUM AMYTAL					WITH SODIUM AMYTAL 10%, 50 MG. PER KG., INJECTED INTRAVENOUSLY					WITH SODIUM AMYTAL, 10%, 150 MG. PER KG., ADMINISTERED ORALLY				
NO. OF ANIMALS	DOSE OF PICROTOXIN IN MG. PER KG.	NO. DIED	NO. SURVIVED	M.L.D. IN MG. PER KG.	NO. OF ANIMALS	DOSE OF PICROTOXIN IN MG. PER KG.	NO. DIED	NO. SURVIVED	M.L.D. OF PICROTOXIN IN MG. PER KG.	NO. OF ANIMALS	DOSE OF PICROTOXIN IN MG. PER KG.	NO. DIED	NO. SURVIVED	M.L.D. OF PICROTOXIN IN MG. PER KG.
3	1	0	3		5	5	0	5		1	2.5	0	1	
4	1.5	1	3		5	6	0	5		1	3.0	0	1	
5	2	2	3		5	7	1	4		1	3.5	0	1	
5	2.5	4	1	2.5	10	8	6	4	8	1	4.0	0	1	
5	3	5	0		5	9	3	2		3	5.0	0	3	
3	4	3	0		3	10	3	0		5	6.0	3	2	6
										3	7.0	3	0	

TABLE III

DETOXIFICATION OF STRYCHNINE SULPHATE BY SODIUM AMYTAL

WITHOUT SODIUM AMYTAL					WITH SODIUM AMYTAL 10% 50 MG. PER KG., INJECTED INTRAVENOUSLY					WITH SODIUM AMYTAL, 10% 150 MG. PER KG., ADMINISTERED ORALLY				
NO. OF ANIMALS	DOSE OF STRYCHNINE IN MG. PER KG.	NO. DIED	NO. SURVIVED	M.L.D. IN MG. PER KG.	NO. OF ANIMALS	DOSE OF STRYCHNINE IN MG. PER KG.	NO. DIED	NO. SURVIVED	M.L.D. OF STRYCHNINE IN MG. PER KG.	NO. OF ANIMALS	DOSE OF STRYCHNINE IN MG. PER KG.	NO. DIED	NO. SURVIVED	M.L.D. OF STRYCHNINE IN MG. PER KG.
3	0.3	0	3		2	1	0	2		1	0.6	0	1	
6	0.4	1	5		3	1.5	0	3		1	0.7	0	1	
5	0.5	0	5		3	2	0	3		1	0.8	0	1	
6	0.6	4	2	0.6	4	2.5	0	4		1	0.9	0	1	
3	0.7	3	0		5	3	0	5		1	1.0	0	1	
					5	4	3	2	4	1	1.1	0	1	
					5	5	4	1		1	1.2	0	1	
										4	1.8	1	3	
											2.4	3	2	2.4
											3.0	3	0	

With sodium amytal subcutaneously the M.L.D. of strychnine is 7 mg. per kg. and intramuscularly the M.L.D. is 5.4 mg. per kg. Thus the subcutaneous and intramuscular injection of sodium amytal did not detoxify cocaine. With picrotoxin and strychnine the subcutaneous and intramuscular injection of sodium amytal produced greater protection than by vein or mouth. This discrepancy by the various routes of administration of sodium amytal is not well understood. It may be partially explained by the relative difference in the rate of absorption of these poisons. The development of toxic symptoms of cocaine is considerably more rapid than with picrotoxin or strychnine. Thus it seems that the intravenous and oral administrations of sodium amytal offers a more consistent prophylactic action for all three poisons.

The use of sodium amytal as an anticonvulsant as well as a detoxifying agent was also studied. The minimal convulsant dose (M.C.D.) of each of the three poisons was determined. The average time of onset of convulsions was also observed for the poisons alone and with sodium amytal. No less than five rabbits were used for each dose.

As shown in Table IV and V the average minimal convulsant dose (M.C.D.) of cocaine alone is 50 mg. per kg. and the average time of onset of convulsions is three minutes. With sodium amytal injected intravenously the average minimal convulsant dose of cocaine is 100 mg. per kg. The average time of onset of convulsions with sodium amytal (intravenously) is 150 minutes. With sodium amytal administered orally the M.C.D. is 60 mg. per kg. and average time of onset of convulsions is fifteen minutes. This shows that sodium amytal injected intravenously is considerably more efficient as an anticonvulsant than when given orally.

The average minimal convulsant dose of picrotoxin is 2 mg. per kg. with the average time of onset of convulsions as twenty minutes. With sodium amytal by vein the M.C.D. of picrotoxin is 5 mg. per kg. and the average time of onset of convulsions is delayed to fifty-four minutes. Orally, sodium amytal prevents convulsions of picrotoxin up to 4 mg. per kg. or twice that of picrotoxin alone. The average time of onset of convulsions is seventy minutes. Thus both the intravenous and oral administrations of sodium amytal increased the convulsant dose to twice that of picrotoxin alone and more than doubled the time of onset of convulsions.

With strychnine alone the average minimal convulsant dose is 0.4 mg. per kg. Sodium amytal by vein increases this dose to 1.5 mg. per kg. or approximately 4 times that of strychnine alone. The average time of onset of convulsions with strychnine alone is eighteen minutes. The average time of onset of convulsions is prolonged to forty minutes with sodium amytal by vein and to thirty-five minutes with sodium amytal by mouth. The average convulsant dose of strychnine is 0.8 mg. per kg. with sodium amytal by mouth. Thus, the threshold for convulsions is raised by intravenous injection and oral administration of sodium amytal. Furthermore, the determination of the average convulsant dose of these poisons is less severe with sodium amytal than with the poisons alone. The convulsant dose of cocaine with sodium amytal is so mild that they are with difficulty noted as convulsions. The drawing back of the head and slight spasms are the only symptoms with cocaine under sodium amytal.

TABLE IV

CONVULSANT DETOXIFICATION OF COCAINE, PICROTOXIN, AND STRYCHNINE BY SODIUM AMYTAL

DRUG	WITHOUT SODIUM AMYTAL						WITH SODIUM AMYTAL 10% 50 MG. PER KG. INJECTED INTRAVENOUSLY						WITH SODIUM AMYTAL 10% 150 MG. PER KG. ADMINISTERED ORALLY					
	NO. OF ANIMALS	DOSE OF DRUG MG. PER KG.	AVERAGE TIME OF ONSET OF CONVULSIONS (MIN.)	NO. HAVING CONVULSIONS	NO CONVULSIONS	M.C.D. MG. PER KG.	NO. OF ANIMALS	DOSE OF DRUG MG. PER KG.	AVERAGE TIME OF ONSET OF CONVULSIONS (MIN.)	NO. HAVING CONVULSIONS	NO CONVULSIONS	M.C.D. MG. PER KG.	NO. OF ANIMALS	DOSE OF DRUG MG. PER KG.	AVERAGE TIME OF ONSET OF CONVULSIONS (MIN.)	NO. HAVING CONVULSIONS	NO CONVULSIONS	M.C.D. MG. PER KG.
Cocaine Hydrochloride	5	30		0	5		5	50		0	5		5	40		0	5	
	5	40	5	2	3		5	75	150	2	3		5	50	15	2	3	
	5	50	3	3	2	50	5	100	150	5	0	100	5	60	10	4	1	60
	5	60	2	5	0													
Picrotoxin	5	1		0	5		3	2		0	3		3	2		0	3	
	10	1.5	25	4	6		5	3		0	5		5	3	90	2	3	
	10	2.0	20	10	0	2.0	5	4	85	2	3		5	4	70	4	1	4
	8	2.5	20	8	0		5	5	54	4	1	5	5	5	40	5	0	
Strychnine Sulphate	5	0.3	20	1	4		5	1.0	50	2	3		5	0.6		0	5	
	5	0.4	18	4	1	0.4	5	1.5	40	3	2	1.5	5	0.7	40	2	3	
	5	0.5	16	5	0		5	2.0	35	5	0		5	0.8	35	4	1	0.8
	8	0.6	15	8	0													

TABLE V

AVERAGE TIME OF CONVULSANT DETOXIFICATION OF COCAINE, PICROTOXIN, AND STRYCHNINE BY SODIUM AMYTAL

DRUG	WITHOUT SODIUM AMYTAL	WITH SODIUM AMYTAL 10%			
	TIME OF CONVULSIONS AFTER INJECTION (MINUTES)	50 MG. PER KG. INJECTED INTRAVENOUSLY		150 MG. PER KG. ADMINISTERED ORALLY	
		TIME OF CONVULSIONS (MINUTES)	AVERAGE DIFFERENCE (MINUTES)	TIME OF CONVULSIONS (MINUTES)	AVERAGE DIFFERENCE (MINUTES)
Cocaine	3	150	147	10	7
Picrotoxin	20	54	34	70	50
Strychnine	18	40	22	35	17

TABLE VI

SUMMARY OF TABLES I, II, AND III. DETOXIFICATION OF COCAINE, PICROTOXIN, AND STRYCHNINE BY SODIUM AMYTAL

DRUG	WITHOUT SODIUM AMYTAL	WITH SODIUM AMYTAL	
	M.L.D. MG. PER KG.	50 MG. PER KG. INTRAVENOUSLY	150 MG. PER KG. ORALLY
		M.L.D. MG. PER KG.	M.L.D. MG. PER KG.
Cocaine	100	400	100
Picrotoxin	2.5	8	10
Strychnine	0.6	4	5.4

TABLE VII

SUMMARY OF TABLE IV. CONVULSANT DETOXIFICATION OF COCAINE, PICROTOXIN, AND STRYCHNINE, BY SODIUM AMYTAL

DRUG	WITHOUT SODIUM AMYTAL	WITH SODIUM AMYTAL	
	M.C.D. MG. PER KG.	50 MG. PER KG. INTRAVENOUSLY	150 MG. PER KG. ORALLY
		M.C.D. MG. PER KG.	M.C.D. MG. PER KG.
Cocaine	50	100 to 400	60
Picrotoxin	2	5	4
Strychnine	0.4	1.5	0.8

With cocaine alone the convulsions are severe. Picrotoxin and strychnine under sodium amytal show less severe convulsions than without sodium amytal but are much more pronounced than with cocaine.

The rate of absorption of these poisons varies if the time of onset of convulsions is an indication. Cocaine shows convulsions in three minutes, whereas, picrotoxin and strychnine produces convulsions in twenty minutes as shown in Tables IV and V. The intravenous route of administration of sodium amytal is rapid in its effect, thus producing greater detoxifying properties for cocaine. The oral use of sodium amytal is much slower in its effect and offers less protection in acute poisoning with cocaine. Sodium amytal by mouth gives greater convulsant protection for picrotoxin and strychnine than for cocaine because of more equal rate of development of their effects.

It is difficult to intimate on which part of the central nervous system sodium amytal offers most protection. The differences observed with poisonous drugs may be ascribed to the nature of the substances studied. Thus among the local anesthetics which all act on the cerebrum, Knoefel, Herwick and Loevenhart[1] showed that sodium amytal does not exhibit the same degree of protection.

During the progress of this study, Maloney, Fitch and Tatum[3] reported the detoxification of sodium amytal by picrotoxin. This is the reverse of our study. In the detoxification of sodium amytal by picrotoxin Maloney, Fitch and Tatum[3] found that approximately twice the M.L.D. of sodium amytal could be given with the picrotoxin treatment. Our findings by the reversible treatment, that is, the detoxification of picrotoxin by sodium amytal was approximately three times the M.L.D. of picrotoxin with sodium amytal.

CONCLUSIONS

1. Protection is afforded by sodium amytal against intoxication of certain poisons that act on the central nervous system.

2. The intravenous and oral administrations of sodium amytal in the order named offers the more effective protection for all three poisons.

3. Sodium amytal is an anticonvulsant as well as a detoxifying agent for the three poisons.

We wish to express our gratitude to Dr. K. K. Chen for suggestions and criticisms.

REFERENCES

1. Knoefel, P. K., Herwick, R. P., and Loevenhart, A. S.: The Prevention of Acute Intoxication from Certain Local Anesthetics, J. Pharmacol. & Exper. Therap. **33**: 265, 1931. Knoefel, P. K., Herwick, R. P., and Loevenhart, A. S.: The Prevention of Acute Intoxication from Local Anesthetics, J. Pharmacol. & Exper. Therap. **39**: 397-411, 1930.
2. Zerfas, L. G., and McCallum, J. T. C.: The Clinical Use of Iso-Amyl-Ethyl Barbiturate, Anesth. & Analg. **8**: 349-359, 1929.
3. Maloney, A. H., Fitch, R. H., and Tatum, A. L.: Picrotoxin as an Antidote in Acute Poisoning by the Shorter Acting Barbiturates, J. Pharmacol. & Exper. Therap. **41**: 465, 1931.

THE PRESENCE AND SIGNIFICANCE OF ISOHEMAGGLUTININS IN THE BODY OUTSIDE THE BLOOD STREAM*

By Leland W. Parr, Beirut, Republique Libanaise

IT IS generally recognized that isohemagglutinins are not confined to the blood stream. They have been found in milk and colostrum,[1] saline extracts of malignant tumors,[2] tissue juices obtained via cantharides blisters,[3] urine,[4] cyst fluids,[5] amniotic fluid,[6] pericardial fluid,[7] and cerebrospinal fluid.[8]

Holzer[7] has recently called attention to the difficulty which is encountered in determining the blood group of a corpse. He suggests that in such cases the pericardial fluid be utilized. Several cubic centimeters of this liquid are always available and it will be found to contain the same isohemagglutinins as occur in the blood stream. It is sometimes desirable to obtain the blood type of a corpse for medicolegal information or to throw light on a fatal transfusion and for such purposes the use of pericardial fluid is suggested.

The presence of antibodies in the milk and colostrum has long been a topic of interest to immunologists, who, in so far as colostrum is concerned, have worked mostly with the bovine species.[9] In these animals the ingestion of colostrum appears to be a more vital matter than in human beings, since in ruminants there is less possibility of placental transmission of antibodies than in an animal such as man which has a simpler placental structure. Heim[1] stated that if the infant were not nursed at the breast the isohemagglutinin content of the serum was affected, agglutinative possibilities being gradually suppressed until in eight to fourteen days the isohemagglutinins would be no more active than in the colostrum, in which viscid medium clear-cut agglutination of red blood cells seldom occurs. On the other hand, Happ[1] did not find more isohemagglutinin in the blood of nursing infants than in that of those artificially fed. It is now well known that the isohemagglutinin content of the blood of the newborn child undergoes changes such as have been indicated by several writers,[10] but these have to do with the maturation of the dynamic specificities peculiar to the child and determined for it by the factors which it has inherited from its father and mother. Only incidentally is the isohemagglutinin content affected by either placental permeability or mother's milk or colostrum. The child may, at first, have some isohemagglutinin derived from the mother; this does not persist, but is replaced by that which its own body has produced. It is being suggested at the present time[11] that somewhat the same thing may happen for a wide variety of protein specificities.

Isohemagglutinins may occur in the cerebrospinal fluid, although as noted by Kolmer[8] they are "only occasionally" so found. Herman and Halber[8] have reported 83 cases in which the cerebrospinal fluid was tested for its isohem-

*From the Department of Bacteriology and Hygiene, American University of Beirut, Beirut, Republique Libanaise.

Received for publication, June 25, 1931.

agglutinin content. They did not find this "antibody" present in normal cases but did find it where there had been organic lesions of the central nervous system in which there was enhanced permeability of the meninges, as in epidemic meningitis, tumor of the spinal cord, and multiple sclerosis. They did not find isohemagglutinins in the cerebrospinal fluid of cases of tubercular meningitis and they noticed that the isohemagglutinin content of the cerebrospinal fluid was lower than that of the blood serum.

At present we regard cerebrospinal fluid as a normal secretion of epithelial origin produced from the choroid plexuses within the ventricles out of which it flows through the foramina of Luschka and Magendie into the subarachnoidal spaces. In the sinuses, however, the cerebrospinal fluid is brought into very close contact with the venous blood, being separated from it in the Pacchionian bodies only by two thin layers, dura and arachnoid. Thus while the normal cerebrospinal fluid is secreted from within the central nervous system itself it may under certain conditions partake of the nature of a transudate.

If it could be established that normal cerebrospinal fluid does not contain isohemagglutinins but that in certain diseased conditions it does (provided the blood does), we would have a simple test of reliability which might conceivably be of considerable aid to the clinician.

It should be pointed out that where the subject in question belongs to the blood group AB (Jansky IV) there are no isohemagglutinins in the blood and there could not be any in the cerebrospinal fluid from a person belonging to such blood group. In our population, however, rather less than 5 per cent of the people fall in blood group AB so that it is evident that this limitation is not a serious one.

While resident in the Near East we were able to test out the isohemagglutinin content of 104 cerebrospinal fluids. In 98 of these fluids no isohemagglutinin could be demonstrated. In 32 of the cases we were only provided with specimens of cerebrospinal fluid and were unable to determine the type of the blood. Of the 72 whose blood was also typed we found 21 to belong to group O (Jansky I), 34 to group A (Jansky II), 7 to group B (Jansky III), and 10 to group AB (Jansky IV). In the Near East somewhat more than 10 per cent of the people belong to group AB which as we have pointed out is more than twice the percentage in America. Six of the cerebrospinal fluids possessed isohemagglutinins and they are summarized in Table I:

TABLE I

	BLOOD GROUP	CEREBROSPINAL FLUID GROUP	HISTORY
Case 1	A	A	Typhoid with meningism; Wassermann reaction negative
Case 2	O	O	Tubercular meningitis; Wassermann reaction negative
Case 3	A	A	Compression myelitis; Wassermann reaction positive
Case 4	B	O	Diabetes mellitus and myelitis; Wassermann reaction negative
Case 5	—	O	No history
Case 6	O	A	Undiagnosed fatal case; Wassermann reaction positive

The cerebrospinal fluid and the other fluids shortly to be mentioned were all typed by the open slide method using known red blood cells to establish the group. In practically all cases the typing was in triplicate and the results clear-cut. Hemolysis of the cells used was not observed.

In discussing the data of the above tabulation three points should be noted. In the first place one of the patients (Case 2) was a patient suffering from tubercular meningitis. Herman and Halber did not find isohemagglutinins in this disease but their paper does not state how many cases of tubercular meningitis they had. It is conceivable that their case or cases belonged to the blood group AB in which event no isohemagglutinins would be found in the cerebrospinal fluid even if there were permeability. Secondly, it will be noted in another one of our cases (6) that the blood showed a typing of group O and the cerebrospinal fluid group A. This would mean that one isohemagglutinin "a" failed to pass into the cerebrospinal fluid. It might be that in some cases there is a differential permeability. Or possibly the missing isohemagglutinogen was absorbed by the tissues. Selective permeability has been mentioned by Hirszfeld and Zborowski[12] and Kritschewski and Schwarzman,[13] among others, have shown that human tissues and organs have the capacity to absorb isohemagglutinins. Thirdly, it will be observed that we have recorded (Case 4) a patient whose cerebrospinal fluid contained the isohemagglutinins "a" and "b" (Type O) whereas only "a" was found in the blood (Type B). This can only be explained on the basis of some error in technic or record and is one of those cases research workers would like to omit from their protocols did their consciences but permit.

The cerebrospinal fluids examined were all from hospital patients in whom there was some reason for an examination of the fluid because of the clinical history and included twelve specimens from insane hospital patients. In these last no isohemagglutinins were found.

We have also examined ten specimens of ascitic fluid, three of hydrocele fluid, two of synovial fluids from inflammatory conditions of the knee joint, and one specimen of hydatid cyst fluid. All of these were typed as of the same group as the blood with the exception of the fluid from the hydatid cyst. In this case there were no isohemagglutinins in the cyst fluid whereas the patient's blood was of group A.

While the isohemagglutinin content of the cerebrospinal fluid is not quantitatively great, that of the other fluids more nearly resembles blood serum in this respect. Thus a hydrocele fluid obtained November 8, 1925, and typed as a strong group B was still strongly reactive and typically group B May 14, 1928, after more than two and a half years of storage.

SUMMARY

The presence of isohemagglutinin is reported for ascitic fluid, hydrocele fluid and for synovial fluid obtained from knee joints the seat of some inflammatory process. No isohemagglutinin could be demonstrated in the one specimen of hydatid cyst fluid studied.

Attention is called to the isohemagglutinin content of the cerebrospinal fluid. It was not present in 94 per cent of our 104 specimens. It is suggested that its

presence may have diagnostic value, to check which further investigation of the point is urged.

REFERENCES

1. Kraus: Wien. klin. Wchnschr. 737, 1901.
Landsteiner: Wien. klin. Rund. No. 40, 1902.
Langer: Ztschr. f. Heilk. **4**: 111, 1903.
Happ: J. Exper. Med. **31**: 313, 1920.
Hara and Wakao: Jahrb. f. Kinderh. **114**: 313, 1926.
Heim: Monatschr. f. Geburtsh. u. Gynäk. **74**: 52, 1926.
Hirszfeld: Konstitutionsserologie und Blutgruppenforschung, Springer, Berlin, 1928.
2. Landsteiner: Wien. klin. Wchnschr. No. 45, 1908.
3. Lenart and König: Klin. Wchnschr. **7**: No. 12, 1928.
4. Friedberger: Berl. klin. Wchnschr. p. 1236, 1900.
Pfeiffer: Ztschr. f. Hyg. **56**: 488, 1907.
5. Brinkerhoff and Southard: J. Med. Res. **9**: 28, 1903.
6. Snyder: Blood Grouping in Relation to Clinical and Legal Medicine, Williams and Wilkins, Baltimore. 1929.
7. Holzer: Klin. Wchnschr. **8**: 2427, 1929.
8. Herman and Halber: Compt. rend. Soc. de biol. **91**: 959, 1924.
Kolmer: Infection, Immunity and Biologic Therapy, Saunders, Philadelphia, 1925.
9. Little and Orcutt: Proc. Soc. Biol. & Med. **19**: 331, 1922, and others.
10. Halban: Wien. klin. Wchnschr. **13**: 545, 1900.
Von Decastello and Sturli: München. med. Wchnschr. **49**: 1090, 1902.
Halban and Landsteiner: München. med. Wchnschr. **49**: 473, 1902.
Langer: Ztschr. f. Heilk. **4**: 111, 1903.
Happ: J. Exper. Med. **31**: 313, 1920.
Deluca: Rev. franç. de gynec. et d'obst. **20**: 331, 1925.
Smith: Am. J. Dis. Child. **36**: 54, 1928.
11. Immunologic Recapitulation, Editorial: J. A. M. A. **96**: 950, 1931.
12. Klin. Wchnschr. **4**: 1152, 1925; Compt. rend. Soc. de biol. **92**: 1253, 1925; ibid. **94**: 205, 1926.
13. Klin. Wchnschr. **6**: 2081, 1927.

THE SIGNIFICANCE OF THE PERIODIC HEALTH EXAMINATION AND ITS INFLUENCE UPON THE HEALTH OF A GROUP OF EXAMINEES*†

Summarizing 500 Consecutive Periodic Health Examinations in Private Practice

By Francis Ashley Faught, M.D., Philadelphia, Pa.

AN IDEAL method of estimating the value of the periodic health examination would be to compare two groups of persons of approximately similar ages and social status. One group to be examined and told of their defects and abnormalities, advised of their importance and instructed regarding their correction, and then reexamined, after a period sufficiently long to permit of the carrying out of the advice given; the other group to be examined and reexamined after a similar interval, but the information obtained withheld, so that any improvement in health would be largely the result of individual initiative in efforts to combat actual disease, or relieve annoying symptoms. Since such an ideal clinical study is impossible, we may, as an alternative, examine a group of apparently healthy persons, outline plans for the improvement of their habits and environment, and the correction of their defects, and then, after a sufficient interval to permit of their accomplishment, reexamine each one and record the improvements in the individual records as shown by the reexamination, compared with the original.

By this method we should be able to show:

1. The relative frequency, number and character of complaints, defects, and abnormalities found.

2. The extent of coöperation of each individual, as shown by a reduction in the number of defects, improvement of habits, and the effect of these upon the health of the individual, as shown by the second examination.

3. The confidence of the public in the periodic health examination as a health-promoting measure; as indicated by the percentage of original examinees returning for reexamination, from which may be determined:

4. The types of abnormalities and defects receiving the greatest attention by the examinees.

5. The age period during which persons show the keenest interest in preserving and improving their health.

Such data was available in the records of five hundred consecutive periodic health examinations performed by me, which furnished the basis for this analysis. They comprise five hundred consecutive health examinations performed in private practice prior to February 1, 1930, and compiled as of February 1, 1931.

*Received for publication, July 1, 1931.

†An abridgement of a paper read before the Philadelphia County Medical Society, as part of a Symposium on The Periodic Health Examination, presented March 25, 1931.

This interval of one year was necessary in order to bring out the elements of the study as outlined above.

DATA RELATING TO 500 CONSECUTIVE PERIODIC HEALTH EXAMINATIONS

In Table I, of sex distribution, it will be noted that a slightly larger number of women were examined than men. This might be interpreted to indicate that women are more interested in maintaining their health than men. In this series the slight preponderance in favor of the female is probably due to the fact that men found it more difficult to keep daytime appointments for examination, or were loath to spare the two hours' time necessary for study.

TABLE I

SEX DISTRIBUTION

Females	284
Males	216
Total	500

In this group the youngest was eighteen and the oldest seventy-four years.

TABLE II

AGES BY TEN YEAR PERIODS

AGE	NO.	PER CENT
18 to 20 years	12	2.4
21 to 30 years	76	15.2
31 to 40 years	138	27.4
41 to 50 years	143	28.6
51 to 60 years	88	18.6
61 to 70 years	35	7.2
Over 71 years	8	1.6
	500	100.0

Table II gives the ages by ten year periods. Here, it will be noted that the majority, 281 or 56 per cent are in the fourth and fifth decades, i.e., between the ages of thirty-one and fifty. This fact strongly suggests the existence of a belief among the laity that this is the time when chronic degenerative processes are prone to develop, so that between these ages, the thoughtful individual begins to show a more active interest in his present health and its future possibilities.

TABLE III

NUMBER AND PERCENTAGE RETURNING FOR REEXAMINATION

One reexamination	112	22.2
Two reexaminations	45	9.0
Three reexaminations	24	6.0
Four reexaminations	30	4.8

Table II sets forth the number who have returned for reexamination one or more times, and indicates a definite appreciation of the value of this health-promoting service, otherwise over 22 per cent would not have been reexamined. The fact that 10.8 per cent have returned three or more times indicates clearly that it is possible to develop, in intelligent persons, what might be called a "health examination habit."

Chart 1 shows the twenty-three principal complaints in the order of their frequency in 431 or 86.2 per cent of this group. The remaining 69 or 13.8 per cent stated definitely in their questionnaires that they did not have any complaints, and that the examination was sought merely as a check-up on their present health.

In addition to the many subjective complaints recorded, there was frequently discovered evidence of faulty habits and bad environment that adversely

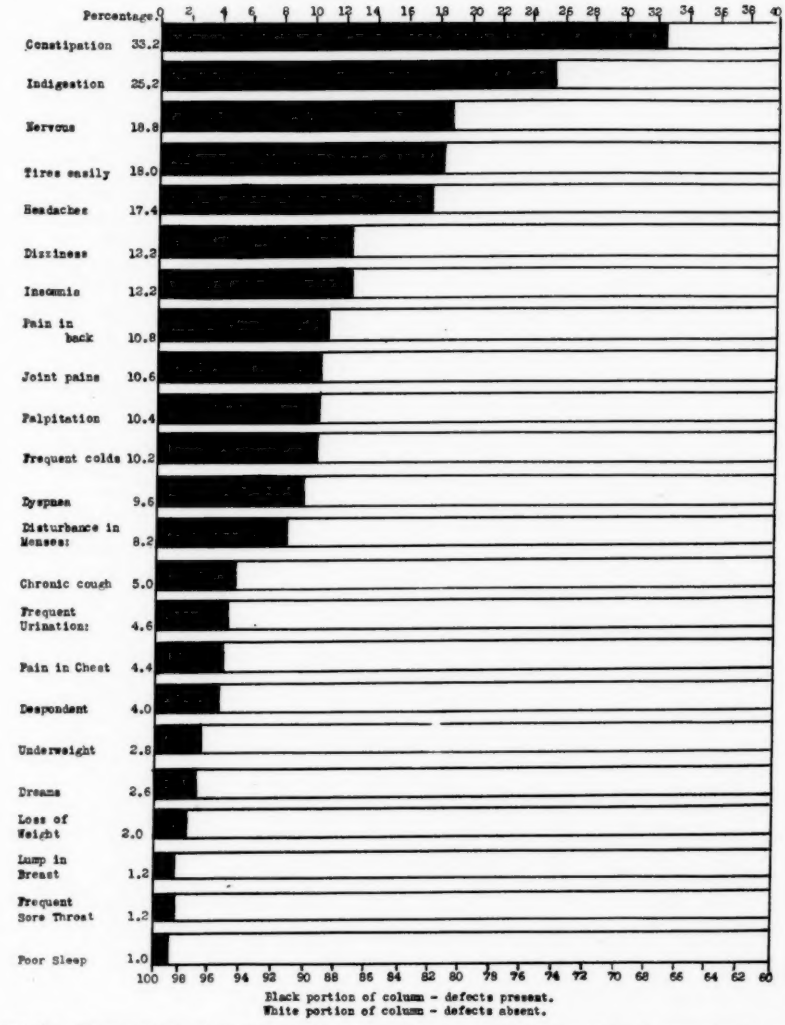


Chart 1.—Percentage occurrence of 23 most commonly mentioned symptoms and complaints in 500 examinations.

influenced the immediate and future health of the individual. The more important of these, in the order of their frequency, and percentage of occurrence, are set forth in Table IV.

In studying Chart 2 which sets forth upon a percentage basis, twenty-five of the principal defects recorded, it should be remembered that this list is far from complete, since it includes neither all abnormalities discovered, nor all that

were reported to the individual at the time of the examination, because it seemed best not to acquaint every individual with the full extent of his defects and abnormalities, as in many instances the report would have been so formidable that it might have discouraged the examinee from doing anything, and therefore, would have defeated the purpose of the examination.

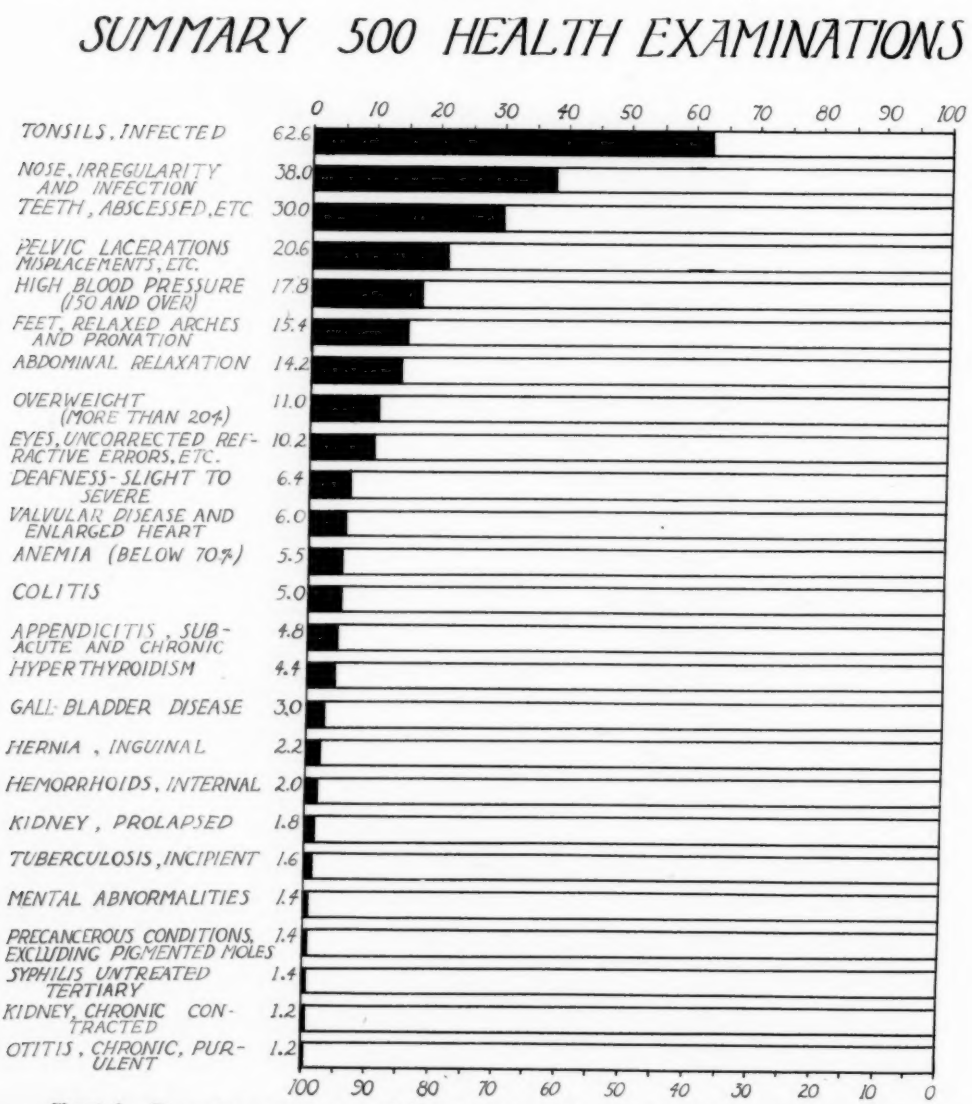

SUMMARY 500 HEALTH EXAMINATIONS
0 10 20 30 40 50 60 70 80 90 100
TONSILS, INFECTED 62.6
NOSE, IRREGULARITY AND INFECTION 38.0
TEETH, ABSCESSED, ETC 30.0
PELVIC LACERATIONS MISPLACEMENTS, ETC. 20.6
HIGH BLOOD PRESSURE (150 AND OVER) 17.8
FEET, RELAXED ARCHES AND PRONATION 15.4
ABDOMINAL RELAXATION 14.2
OVERWEIGHT (MORE THAN 20%) 11.0
EYES, UNCORRECTED REFRACTIVE ERRORS, ETC. 10.2
DEAFNESS - SLIGHT TO SEVERE 6.4
VALVULAR DISEASE AND ENLARGED HEART 6.0
ANEMIA (BELOW 70%) 5.5
COLITIS 5.0
APPENDICITIS, SUBACUTE AND CHRONIC 4.8
HYPERTHYROIDISM 4.4
GALL-BLADDER DISEASE 3.0
HERNIA, INGUINAL 2.2
HEMORRHOIDS, INTERNAL 2.0
KIDNEY, PROLAPSED 1.8
TUBERCULOSIS, INCIPIENT 1.6
MENTAL ABNORMALITIES 1.4
PRECANCEROUS CONDITIONS, EXCLUDING PIGMENTED MOLES 1.4
SYPHILIS UNTREATED TERTIARY 1.4
KIDNEY, CHRONIC CONTRACTED 1.2
OTITIS, CHRONIC, PURULENT 1.2
100 90 80 70 60 50 40 30 20 10 0

Chart 2.—Percentage occurrence of 25 most frequently recorded defects and abnormalities recorded in 500 health examinations.

The actual amount of evidence suppressed depended, in a large measure, on the number of defects and abnormalities met in each particular individual, so that even as here recorded it must not be assumed that active intervention was advised in all instances when defects were noted. In this study none were found free of defects, the average number per person was 2.86 and the largest number in any one person was 9.

The criterion applied to determine the inclusion of abnormalities and defects was primarily whether the condition, as discovered, appeared directly or indirectly to affect the individual's immediate health, and comfort, or whether if uncorrected, or unarrested, they would, in a reasonably short time, result in disease, or be the cause of discomfort or disability. It is important that this fact be borne in mind, otherwise there may be some surprise at the relatively small number of defects noted.*

TABLE IV

SOME DETRIMENTAL HABITS REQUIRING MODIFICATION

FINDINGS	NO.	PER CENT	
1. Insufficient exercise	109	20.1	
2. Careless diet habits	63	12.6	
3. Insufficient sleep, under 6 hours	39	7.8	
4. Insufficient fluid intake, less than 50 ounces in 24 hours	28	5.6	
5. Excessive use of tobacco		20.0	males only
6. Excessive use of coffee and tea	25	5.0	
7. Long working days	19	3.8	
8. No recent vacation	18	3 6	
9. Excessive hours of work (weekly)	13	2.6	

Commenting upon the percentage of chronically infected tonsils found, it should be stated that this does not represent the total of infected tonsils originally possessed by this group, because it was ascertained at the time of examination that 75 or 15 per cent had previously submitted to tonsillectomy, so it may be concluded that there were in this series, originally 78.6 per cent of persons with infected tonsils.

THE DATA OF 70 COMPARATIVE EXAMINATIONS

In selecting the records of patients who returned for examination, it was found that only seventy, of the one hundred and twelve noted in Table III, were available for purposes of comparison, because in the earlier examinations, the information recorded was not sufficiently complete to furnish a basis for comparative analysis, so that it is upon this comparatively small series that the discussion which follows is based.

The average interval between examinations was 18.8 months; the shortest eight months, the longest fifty months.

Chart 3 shows, on a percentage basis, the 15 most frequently occurring defects and abnormal physical findings recorded in this group. This chart should be compared with Chart 4, in which appears in the same order, the percentage of abnormalities corrected during the interval between the first and second examinations.

In addition, a number of less frequently occurring defects and abnormalities, and a few special examinations were, when advised, corrected, treated, or investigated in each instance. These included: one each of myxedema, internal hemorrhoids, perirectal abscess, gallstones, chronic otitis media and ureteral calculus; also, one changed an indoor sedentary occupation for one with a maximum

*It should also be noted that many of these health examinations were performed on patients previously under observation and in whom, in many instances, major corrections had already been made.

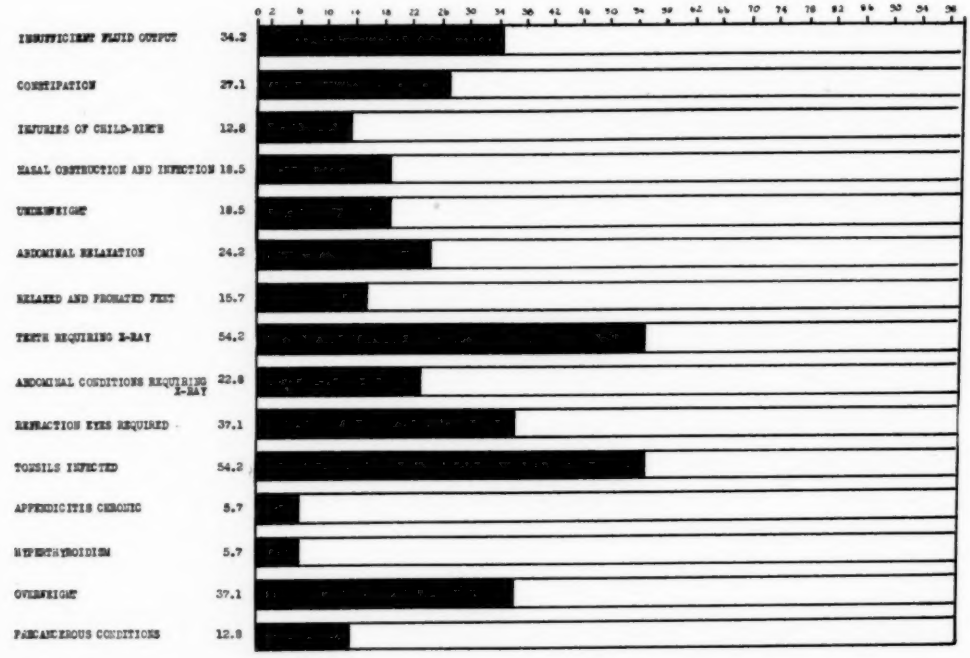


Chart 3.—Percentage occurrence of 15 most frequently occurring defects and abnormalities found at first examination.

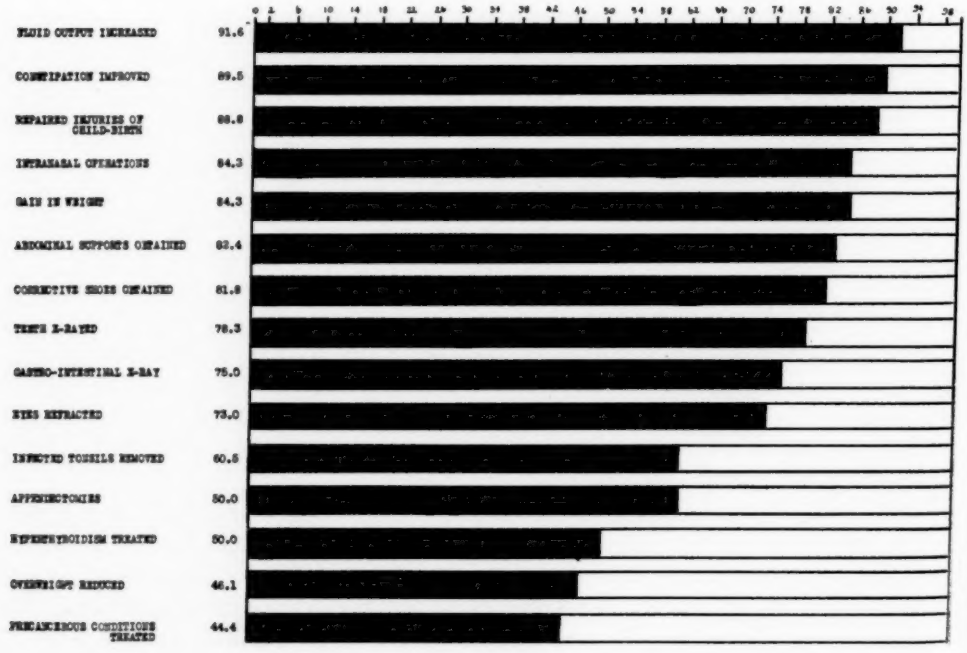


Chart 4.—Percentage of defects corrected, as noted at time of first reexamination.

of fresh air and sunshine; two patients with inguinal hernia adopted the wearing of a truss as an alternative to radical operation; one had an x-ray of the chest, and one had an involved joint x-rayed; two were cystoscoped, and there were ten patients treated for anemia (hemoglobin less than 70 per cent), and fourteen had impacted cerumen removed.

As a result of their original examination, nine were recommended to have obviously diseased teeth removed, while a total of twenty-nine were advised to have a partial or complete dental x-ray. As a result of the x-ray findings, a total of nineteen submitted to single, or multiple extractions, as a result of which, fourteen patients, finding themselves with insufficient teeth to properly masticate their food, obtained artificial restoration.

An unexpected by-product was the apparent effect of generally improved health of the group upon abnormalities in blood pressure. There were encountered twenty-five persons whose systolic blood pressure was definitely above the normal average for their age. At the reexamination, seventeen showed a distinct lowering in high pressure, the average reduction being 20 mm. Hg., the greatest in any one individual was 55 mm. Hg. There were also recorded seven cases of hypotension, four of whom showed a definite rise to approximately normal at the time of reexamination. Since none of these individuals at the time of their first examination had alarmingly high pressure, no specific advice or treatment was directed toward its reduction, so that it is rather interesting to note that abnormalities of blood pressure tend, without specific treatment, to approximate normal, in proportion as the general health of the individual improves, as for example, through the institution of corrective and remediable measures growing out of a periodic health examination.

SUMMARY AND CONCLUSIONS

An analysis of five hundred consecutive periodic health examinations is presented, together with 22.2 per cent reexaminations made after a period of one year or longer, 10.8 per cent of these having been reexamined three or more times during a period of about five years.

There were slightly more females than males in the series; 56 per cent of the total were between the ages of thirty-one and fifty years; 13.8 per cent presented for examination with no complaints, but all were found to have one or more defects. The average was 2.86 per person, the greatest number of defects in one individual was 9. A tabulation of the more commonly occurring complaints is presented in order of frequency and percentage of occurrence, together with the twenty-five most common physical abnormalities found, presented in similar form.

A smaller group comprising those who returned for one reexamination is analyzed to show the order of frequency and percentage of occurrence of complaints and abnormalities, and in addition, the percentage of corrections of abnormalities originally reported as determined at the reexamination, from which may be concluded that:

1. The periodic health examination definitely appeals to the public, as a means of discovering and arresting those conditions that may later lead to physical infirmity.

2. Females and males are about equally interested in the promotion and prolongation of their health.

3. The age of greatest interest in matters of health lies between thirty-one and fifty years.

4. No adult, upon careful examination, will be found entirely free of physical defects, or without bad habits, and adverse conditions of environment.

5. A surprising number of physical defects and abnormalities and detrimental habits may be discovered in apparently healthy persons by means of the periodic health examination.

6. Properly presented, there seems to be little difficulty in persuading the individual of average intelligence to proceed with corrective treatment, even when it entails special examinations, radical operations and prolonged treatment.

7. There is a distinct tendency toward improvement in hypertension and hypotension, following the removal and correction of abnormalities and defects discovered at the time of examination.

8. There can be very little doubt that the systematic discovery of defects and abnormalities and corrections can fail to lead to better health, improved efficiency and longer life.

5006 Spruce Street

THE ISOLATION OF AN ORGANISM OF THE ABORTUS-MELITENSIS GROUP FROM A BLOOD CLOT, THE SERUM OF WHICH FAILED TO GIVE AGGLUTINATION WITH B. ABORTUS*†

By Ruth Gilbert, M.D., and H. Gladys Dacey, B.S., New York, N. Y.

FAILURE to obtain an agglutination reaction with B. abortus in the sera of patients having undulant fever has been reported by a number of authors. Only two, however, have mentioned the isolation of the organisms from the blood in such cases. Montagnani[1] (1923), in an article on the intradermal reaction, referred to early cases of undulant fever in which no agglutinative properties could be demonstrated in the serum although the organisms were isolated from the blood and an intradermal reaction was obtained. Burnet[2] (1925) stated that, in his experience, the absence of the agglutination reaction was more frequent in the sera of patients infected with B. melitensis than in those infected with B. abortus. Tramontano[3] reported the results of the study of sixty-five patients having clinical symptoms of undulant fever, the sera from nine of whom failed to give agglutination with B. abortus. He mentioned, however, that ten of the sixty-five cases proved not to be undulant fever. Carpenter and Boak[4] (1930) indicated that frequently no agglutinative properties are present in the serum of patients suffering from the disease. They mentioned that 6 per cent of the cases which they had studied had fallen into this group. In a personal communication, however, Carpenter stated that he had isolated the organism from the blood of only three of these patients. Giordano and Sensenich,[5] in discussing the literature relative to cases of undulant fever where the blood fails to agglutinate the incitant of the infection, make the following statement: "Our cases suggest this possibility but further investigation of the point is necessary."

Since B. abortus has so seldom been isolated from the blood of such cases, the report of an additional instance may be of interest.

A specimen of clotted blood was submitted to the branch laboratory three weeks after the onset of illness. No agglutination of B. abortus was obtained either in this serum or in one examined two weeks later in the central laboratory in Albany. Tests were also performed with B. typhosus, B. paratyphosus A, and B. paratyphosus B without agglutination being obtained. The blood clot was cultured in about 25 c.c. of liver-infusion broth, P_H 6.8, in a bottle with a rubber stopper and incubated at 37° C. for one week. At the end of that time, two plates of liver-infusion agar were inoculated with the broth and incubated in a closed jar in which approximately 10 per cent of the air had been replaced by carbon dioxide. After one week's incubation, colonies of organisms belonging to the abortus-melitensis group had developed. Suspensions of the growth were

*From the Division of Laboratories and Research, New York State Department of Health, Branch Laboratory, New York.
Received for publication, August 1, 1931.
†Presented at the meeting of the New York State Association of Public Health Laboratories, Albany, November 7, 1930.

agglutinated in a 1:2500 dilution of immune serum prepared with B. abortus. The organisms were not agglutinated in the patient's serum.

The history which accompanied the specimen gave the following data. The patient, a housewife of forty-five years, became ill on April 1, 1930, with pain in the chest, headache, vomiting, shortness of breath, and fever varying from 100 to 103°. She was delirious at times. There was no history of diarrhea, constipation, or arthritis. Remissions of temperature occurred every day at about noon, lasting for approximately four hours. The pulse varied from 88 to 120, respiration from 24 to 40. The spleen was not palpable and the lymph glands were not enlarged. In April, 1928 (two years before), the patient had been ill for eighteeen weeks, the symptoms noted at that time corresponding exactly with those observed in 1930.

There was no history of contact with goats, cows, or swine, but the patient had drunk raw milk obtained from a herd of cows in which abortions had occurred.

REFERENCES

1. Montagnani, D. M.: L'intradermoreazione nel tifo, paratifo A e B e nello febbre melitense con i filtrate di cultura, Riv. Crit. di Clin. Med. **24**: 149, 1923.
2. Burnet, E.: Sur la notion de paramelitensis, Arch. Inst. Pasteur de Tunis **14**: 247, 1925.
3. Tramontano, V.: Il valore della siero agglutinazian e della emocultura per in diagnosi di militense, Gazz. Internaz. Med.-chir. **30**: 226, 1925.
4. Carpenter, C. M., and Boak, R. A.: The laboratory diagnosis of undulant fever, J. LAB. & CLIN. MED. **15**: 437, 1930.
5. Giordano, A. S., and Sensenich, R. L.: Brucella abortus infection in man J. LAB. & CLIN. MED. **15**: 421, 1930.

NORMAL RELATIONSHIPS OF BLOOD AND URINE PHOSPHORUS*

By Burnham S. Walker, Ph.D., Boston, Mass.

THE relationships of excretion rates of certain urinary constituents to the amounts simultaneously found in the blood have been extensively studied. This is particularly true of urea, for which substance several investigators have proposed mathematical formulations representing the average excretion rates for definite blood levels (Ambard, 1920; Addis and Watanabe, 1917; McLean, 1915; Austin, Stillman and Van Slyke, 1924; Rabinowitch, 1925; Adolph, 1925; Walker and Rowe, 1927). The relationships observed by these different investigators, while not identical, are in no case widely divergent. Such differences as occur are to be attributed rather to varying approach to the mathematical analysis of the data obtained, than to fundamental differences in the observations themselves. It is possible, by taking into consideration variations in the experimental procedure, to harmonize the results obtained in the case of urea.

This does not seem to be the case as far as the other urinary constituents are concerned, except perhaps in the case of creatinine, where we are dealing with what is for all practical purposes a constant blood level and a constant excretion rate. Surely in the case of phosphorus, the problem is as yet by no means clear. This paper is a report of an attempt to observe the normal variations in the excretion of phosphorus and to compare them with the varying content of the blood in the various groups of phosphorus compounds. The general experimental procedure is based on that used in a previous study of urea excretion (Walker and Rowe, 1927) which in turn was based upon the methods of McLean (1915).

Subjects.—The observations were made upon sixty studuents in the first year of their medical course. All were in good health, and had shown satisfactory kidney function with the phenolsulphonephthalein test and by analysis of the blood for the usual nitrogenous constituents. Five of the group were women; no differences in phosphorus excretion attributable to sex were noted, hence they have been included in the group without special designation.

Collection of Samples.—With the subject fasting, a one-hour urine collection, accurately timed, was completed before 9:00 A.M. Water was allowed as desired by the subject; since this was a study of variations, no attempt was made to standardize water intake or activity. Subjects were permitted to occupy themselves as they wished during the hour of collection. Blood was taken at approximately the middle of the hour period, placed in a bottle containing dry lithium oxalate (one mg. per c.c. blood) and the analysis started at once.

Analytical Methods.—In the blood, determinations were made on each sample for inorganic phosphate phosphorus, acid soluble phosphorus, and total phosphorus, using the methods of Fiske and Subbarow (1925) with such slight additions and modifications as described by Walker and Huntsinger (1930). From these analytical results, values were obtained for inorganic phosphate phosphorus,

*From the Evans Memorial and Boston University School of Medicine.
Received for publication, August 15, 1931.

acid-soluble organic phosphorus, total phosphorus and lipin phosphorus. The last was obtained by subtraction, rather than by direct analysis (Kay and Byrom, 1927; Walker and Huntsinger, 1930).

In the urine, aside from measurement of the volume of the hour collection, determinations were made of inorganic phosphate phosphorus by the method of Fiske and Subbarow (1925) and of total phosphorus. This latter analysis was carried out as follows: a measured volume of urine (the same volume as taken for inorganic phosphate determination; usually one c.c., occasionally up to five c.c.) was put into a 200 × 25 mm. pyrex test tube and five c.c. of 10 normal sulphuric acid added. The water was driven off by heating in a beaker of boiling water until charring took place. The remaining acid solution was heated over a micro-burner with the addition of strong nitric acid drop by drop until the solution was clear and colorless, and for about thirty seconds longer. It was then cooled and transferred to a 100 c.c. volumetric flask with about 60 c.c. of water.

To the contents of the flask was added 10 c.c. Molybdate III (Fiske and Subbarow), four c.c. of the usual reducing agent (1-2-4 amino-naphthol sulphonic acid) and water to 100 c.c. volume. After mixing and allowing to stand ten minutes, a reading was made in the Duboscq colorimeter against the usual standard (0.4 mg. P). It will be noted that this procedure is the same as that for total phosphorus in whole blood. It requires less time for the digestion on account of the smaller amount of organic matter present.

The difference, if any, between the inorganic and total phosphorus represents the organic phosphorus in the urine. This method of determination of organic phosphorus by difference is of course subject to the added errors of the two component analyses. A preferable method for larger amounts of urine would be that described by Youngburg and Pucher (1924), in which the inorganic phosphate is first removed by precipitation with magnesia mixture and the filtrate digested. This method requires larger samples than are often obtained in hour specimens.

Inorganic Phosphate in the Urine.—The inorganic phosphate varied between a minimum of 0.0256 mg. (as P) per c.c. urine and a maximum of 2.10 mg. per c.c., with a mean of 0.603 mg. per c.c. The median value is slightly lower, 0.522 mg. per c.c.

The maximum of 2.10 mg. per c.c. of urine does not in any way represent a "maximum concentration" in the sense used by Ambard, meaning the limit to which the kidney is able to concentrate a given substance. Wigglesworth and Woodrow (1924), by taking sodium phosphate by mouth reached urine concentrations of 3.7 mg. per c.c., and did not feel that they had reached a limit to the power of the kidney to concentrate phosphate.

Organic Phosphorus in the Urine.—The occurrence of organic compounds of phosphorus in the urine is still, despite a massive number of titles on the subject, frequently denied or neglected in books or papers dealing with urinary phosphorus. The weight of the evidence appears to indicate the presence of an appreciable amount of phosphorus in an unoxidized form. The literature up to 1914 is summarized by Forbes and Keith (1914) who state that the organic phosphorus fraction is too large a factor to ignore in any quantitative work.

Youngburg and Pucher (1924) have made an extensive study of the excretion of organic phosphorus, using their direct method of analysis. They find an average elimination of 0.131 mg. (as P) per kilo per twenty-four hours.

Brain, Kay and Marshall (1928) found organic phosphorus in the urines of 17 normal individuals in concentrations ranging from 0 to 0.028 mg. per c.c. using Briggs' method.

The possible pathologic significance of increased organic phosphorus in the urine has been considered occasionally since the time of Zuelzer (1881) and of Lépine, Eymonnet and Aubert (1884). Symmers (1904-05) studied the excretion of organic phosphorus in the urine in nine different diseases, finding it significantly increased in lymphatic leucemia and in nervous diseases.

In our series of normal individuals, organic phosphorus determinations in the urine were made in 59 cases. In 15 of these no organic phosphorus was detectable. In the remaining 44 cases the organic phosphorus varied to a maximum of 0.195 mg. per c.c. The mean value (including all cases) was 0.018 mg. per c.c.

The hour's excretion of organic phosphorus varied up to 4.2 mg., with a mean value of 0.729 mg. Assuming a constancy of excretion throughout the twenty-four hours (which is not only unlikely, but distinctly opposed to the finding of an irregular excretion rate for organic phosphorus (Youngburg and Pucher, 1924), yet convenient for a rough comparison of results) the twenty-four-hour output of organic phosphorus would be up to 100 mg. This is a lower value than that observed by Mathison (1910), and is in fair agreement with the figure obtained by Youngburg and Pucher (1924) and quoted above. No correlation between the amount of organic phosphorus eliminated and the blood levels of any of the groups of phosphorus-containing substances was noted.

Excretion Rates of Inorganic Phosphorus.—With hourly urine volumes ranging from six to 350 c.c., the amount of inorganic phosphate (as P) excreted during the hour varied from a maximum of 63.7 mg. to a minimum of 4.44 mg. The mean value for the hour's excretion was 21.7 mg., the median 18.6 mg.

The simultaneously observed concentration of inorganic phosphorus in the blood in these same cases varied from 2.6 to 4.7 mg. per 100 c.c. whole blood. The mean value was 3.6 mg.

The correlation coefficient between concentration of inorganic phosphate in the blood and the rate of excretion was 0.416 ± 0.071 showing that the effect of varying blood concentration on output is apparently less than is the case with urea, where we have shown that the correlation coefficient is 0.840 ± 0.013 (Walker and Rowe, 1927).

The linearity of regression in the case of phosphorus can be demonstrated by calculation of the correlation ratios

$$\eta\ yx = 0.434$$
$$\eta\ xy = 0.398$$

which do not vary from the correlation coefficient by more than its probable error.

By plotting the average values of inorganic phosphate output per hour for each blood phosphate level (using intervals of 0.2 mg.), we obtain the points shown in Fig. I. Assuming a threshold for inorganic phosphate at a blood level

of 2.4 mg. per 100 c.c. (Wigglesworth and Woodrow, 1924) the points are well represented by a line (drawn solid) the equation of which is

$$D = 18.2\ (B\text{-}2.4)$$

where D is the output of inorganic phosphate in mg. per hour and B is the concentration of inorganic phosphate in the blood in mg. per 100 c.c.

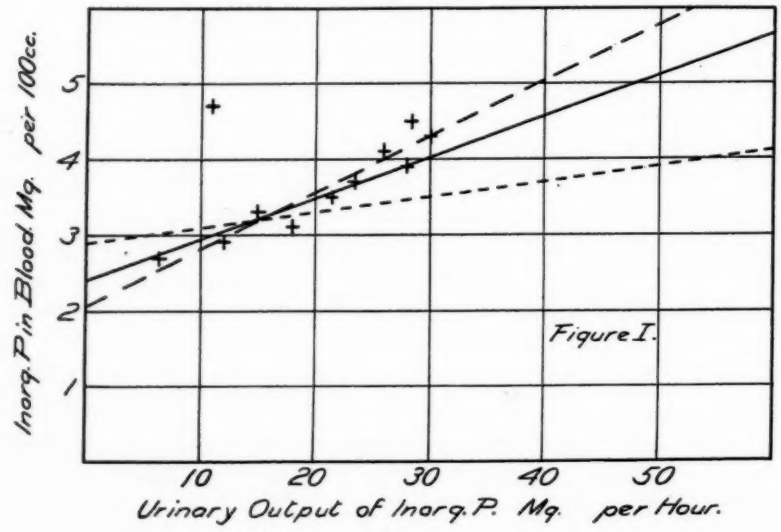
The line drawn in dashes represents the best fitting line regardless of the threshold. This line intercepts the x-axis at about 2.1, fixing the threshold at that point, and giving a somewhat steeper slope. The equation is

$$D = 13.5\ (B\text{-}2.1)$$

The dotted line represents the equation

$$D = 50\ (B\text{-}2.9)$$

derived by Adolph (1925) from the data of Wigglesworth and Woodrow. In this experiment water was taken in excess. Under such conditions the ratio be-

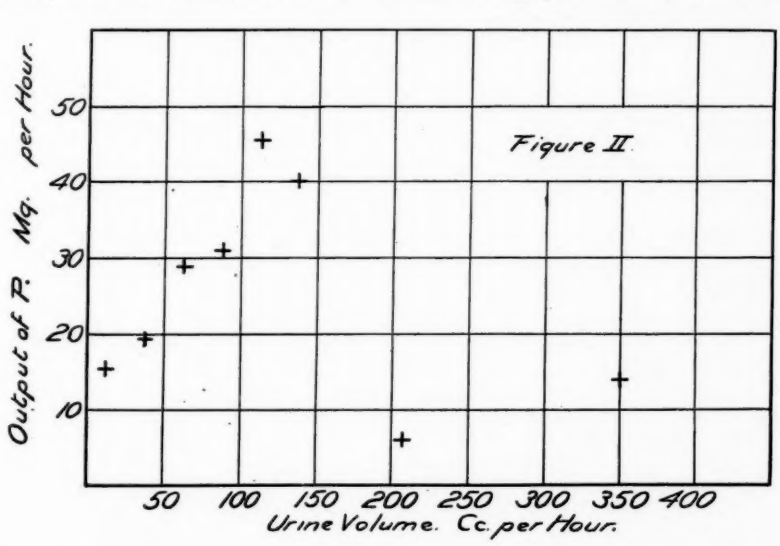


tween the blood concentrations and the elimination rates of urea tends to be stabilized (Addis and Drury, 1923). It seems probable that the difference between the two lines is attributable to the difference in water elimination between our experiments and those of Wigglesworth and Woodrow, and that with increased water output our line would approach the other.

The effect of urine volume on the excretion rate of inorganic phosphate within the limits of variation found in our series seemed at first to be negligible. The correlation coefficient between excretion rate and urine volume was 0.191, a value which can properly be considered as representing no correlation. If the average output of inorganic phosphate be plotted for each urine volume (using intervals of 25 c.c.) the points shown in Fig. II are obtained. This is suggestive of the existence of an "augmentation limit" for phosphate similar to that defined for urea by Austin, Stillman and Van Slyke (1921). The correlation appears to be fair up to about 100 c.c. and then breaks down completely. Previous

investigators (Wigglesworth and Woodrow, 1924; Havard and Reay, 1926; Brain, Kay and Marshall, 1928) have observed the failure of correlation of output with volume: all of these investigators have worked with large volumes of water. It seems definitely established that beyond a certain maximum the amount of water eliminated has no effect on the rate of phosphorus excretion. Below 100 c.c. per hour there seems to be a limiting effect of small volume on rate of elimination.

Most modern workers agree to the existence of a threshold for phosphate (see Adolph, 1925). Failure of excretion of phosphate below the threshold has not been demonstrated in man. Brull (1927) found that under chloralose anesthesia dogs ceased to eliminate phosphate below a blood level of 4 mg. per 100 c.c. plasma. The threshold suggested by Wigglesworth and Woodrow for man (2.4 mg. per 100 c.c. blood) coincides well with our observed excretion rates, which allow for a threshold between 2.0 and 2.4 mg. per 100 c.c. whole blood.



Other factors than concentration of inorganic phosphate in the blood and rate of water elimination undoubtedly influence the rate of phosphate excretion. The concentration of acid-soluble organic phosphorus compounds in the blood might be expected to have an effect as a result of the action of kidney phosphatase (Brain and Kay, 1929). Since, however, the major portion of this group of compounds is in the corpuscles (Brain, Kay and Marshall, 1928), one would hardly expect correlation between whole blood values and urine output. Increase of the ester phosphorus of the plasma by injection of sodium glycerophosphate (Brain and Kay, 1929) conditions an increase in the elimination of inorganic phosphate. In our series, we found no correlation between the acid-soluble organic phosphorus of the whole blood and the rate of elimination of inorganic phosphate. The same was true of the lipin phosphorus.

Another variable which we have not considered in this series is the state of the alkaline reserve. With increased necessity for the elimination of acid, Haldane, Wigglesworth and Woodrow (1924) have shown that there is an increase in the elimination of phosphate.

SUMMARY

In a series of 60 young adults, under normal conditions in all respects except that they were fasting:

1. The inorganic phosphate of the urine varied between 0.0256 and 2.10 mg. per c.c., with a mean of 0.603 mg. (all values given as P).

2. The organic phosphorus of the urine varied between 0 and 0.195 mg. per c.c., with a mean of 0.018 mg.

3. With urine volumes varying from 6 to 350 c.c. per hour (a) the hourly elimination of inorganic phosphate varied from 4.44 mg. to 63.7 mg., mean value 21.7 mg., (b) the hourly elimination of organic phosphate varied from 0 to 4.2 mg., with a mean at 0.729 mg.

4. The correlation coefficient between concentration of inorganic phosphate in the blood and the rate of elimination of inorganic phosphate was 0.416 ± 0.071.

5. The threshold value for inorganic phosphate lies between 2.4 mg. per 100 c.c. whole blood (as suggested by Wigglesworth and Woodrow) and 2.0 mg.

6. While there is no correlation between water excretion and phosphorus excretion over the whole series, there seems to be a tendency for simultaneous increase up to an "augmentation limit" at a urine volume of about 100 c.c. per hour.

7. No correlation was observed between excretion rates of inorganic or organic phosphorus and the blood levels of the groups of organic phosphorus compounds.

REFERENCES

1. Addis, T., and Watanabe, C. K.: The Rate of Urea Excretion, III. The Effect of Changes in Blood Urea Concentration on the Rate of Urea Excretion, J. Biol. Chem. **29**: 391-98, 1917.
2. Addis, T., and Drury, D. R.: The Rate of Urea Excretion. V. The Effect of Changes in Blood Urea Concentration on the Rate of Urea Excretion, J. Biol. Chem. **55**: 105-11, 1923.
3. Adolph, E. F.: The Chemical Sensitiveness of the Kidneys, Am. J. Physiol. **74**: 93-110, 1925.
4. Ambard, L.: Physiologie normale et pathologique des reins, ed. 2, Paris, 1920, Masson et Cie.
5. Austin, J. H., Stillman, E., and Van Slyke, D. D.: Factors Governing the Excretion Rate of Urea, J. Biol. Chem. **46**: 91-112, 1921.
6. Brain, R. T., and Kay, H. D.: A New Test of Renal Function, Quart. J. Med. **22**: 203-16, 1929.
7. Brain, R. T., Kay, H. D., and Marshall, P. G.: Observations on Phosphates in Blood and on the Urinary Excretion of Phosphates, Biochem. J. **22**: 628-48, 1928.
8. Brull, L.: Seuil d'excretion des phosphates minéraux, Compt. rend. soc. de biol. **97**: 731-3, 1927.
9. Fiske, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus, J. Biol. Chem. **66**: 375-400, 1925.
10. Forbes, E. B., and Keith, M. H.: Phosphorus Compounds in Animal Metabolism, Technical Bull. No. 5, Ohio Agricultural Exp. Station, Wooster, Ohio, 1914.
11. Haldane, J. B. S., Wigglesworth, V. B., and Woodrow, C. E.: The Effect of Reaction Changes on Human Inorganic Metabolism, Proc. Roy. Soc. **96B**: 1-14, 1924.
12. Havard, R. E., and Reay, G. A.: Note on the Excretion of Phosphate During Water Diuresis, Biochem. J. **20**: 99-101, 1926.
13. Kay, H. D., and Byrom, F. B.: Blood Phosphorus in Health and Disease I. The Distribution of Phosphorus in Human Blood in Health, Brit. J. Exper. Path. **8**: 429-36, 1927.
14. Lépine, R., Eymonnet, and Aubert: Sur la proportion de phosphore incomplètement oxydé contenue dans l'urine, spécialement dans quelques états nerveux, Compt. rend. Acad. d. sc. **98**: 238-41, 1884.

15. Mathison G. C.: The Output of Organic Phosphorus in Urine, Biochem. J. **4:** 274-9, 1909.
16. McLean, F. C.: The Numerical Laws Governing the Rate of Excretion of Urea and Chlorides in Man. I. An Index of Urea Excretion and the Normal Excretion of Urea and Chlorides, J. Exper. Med. **22:** 212-36, 1915.
17. Rabinowitch, I. M.: Urea Tests of Renal Efficiency, J. Biol. Chem. **65:** 617-22, 1925.
18. Symmers, D.: A Contribution to the Knowledge of the Excretion of Organic Phosphorus in the Urine in Certain Pathological Conditions, J. Path. & Bact. **10:** 159-72, 1904.
19. Symmers, D.: An Additional Note on the Excretion of Organic Phosphorus in the Urine, J. Path. & Bact. **10:** 427-30, 1905.
20. Walker, B. S., and Huntsinger, M. E.: The Phosphorus Partition in Normal Whole Blood, J. Lab. & Clin. Med. **16:** 247-52, 1930.
21. Walker, B. S., and Rowe, A. W.: Renal Excretion With Special Reference to Ambard's Laws, Am. J. Physiol. **81:** 738-54, 1927.
22. Walker, B. S., and Rowe, A. W.: The Relation of Blood to Urine Urea, Am. J. Physiol. **81:** 755-64, 1927.
23. Wigglesworth, V. B., and Woodrow, C. E.: The Relation Between the Phosphate in Blood and Urine, Proc. Roy. Soc. **95B:** 558-70, 1924.
24. Youngburg, G. E., and Pucher, G. W.: Analytical Methods and Observations on the Organic Phosphorus of the Urine, J. Biol. Chem. **62:** 31-44, 1924.
25. Zuelzer, W.: Die klinische Bedeutung der Phosphorsäure des Harns. Trans. Internat. Med. Congress, 7th session, London, 1881 (Vol. II).

LABORATORY METHODS

THE ASCHHEIM-ZONDEK TEST, MODIFIED, FOR DIAGNOSIS OF EARLY PREGNANCY*

Clinical Application in 100 Cases

By John I. Fanz, M.D., and Edwin S. Gault, M.D., Philadelphia, Pa.

OUR work in the corroboration of the original Aschheim-Zondek test was begun early in February, 1929, and at that time the original method[1] was closely adhered to. After the performance of possibly a dozen tests, the results of which are not included in this article, we were clearly convinced that for routine work Aschheim's idea of using varied amounts of urine in 5 mice was unnecessary and impractical. The authors, in their original article, frown upon any modification of their technic, but nevertheless we believe that safe results are always obtained with the use of 0.3 c.c. of the patient's urine, provided it be secured as a first specimen when the patient arises in the morning. If this specimen is filtered and refrigerated, and kept refrigerated, during the three days' duration of the test, it is always well tolerated by the mice, and will give clear-cut results.

The objects of this investigation can be summarized as follows:

1. To verify the high degree of accuracy claimed by Aschheim and Zondek.
2. To present a series of cases including pregnant females, nonpregnant females, and male controls.
3. To establish the earliest possible date at which the test is reliable.

1. Accuracy.—In our series of 100 cases, 6 were fallacious, giving us an accuracy of 94 per cent. Of the 6 cases in error, the first and second, series Nos. 2 and 87, were questionably positive, and repetition was advised. This was possible in the first case, and resulted in a correct reaction. The other (second case) passed from our observation before the test could be repeated. In the third case, series No. 80, the urine was apparently toxic, and resulted in the death of 3 of the 5 mice, and the test was positive in only one ovary. In the fourth and fifth cases, series Nos. 38 and 41, the reactions were clear-cut and the discrepancy cannot be explained at the present writing. In the sixth case, series No. 95, a negative gross and histologic result was obtained; the clinically positive diagnosis, however, depending in this case upon the surgeon's opinion of early pregnancy during laparotomy, no follow-up was possible.

It will therefore be seen that the accuracy of the test is probably as high as 98 per cent.

In no case was a positive reaction obtained on male control urine. One case was positive in a nonpregnant female. Five cases were negative in pregnant

*From the Laboratories of Pathology, Medical School, Temple University.
Received for publication, August 8, 1931.

women. In none of these five was the sample the first morning specimen, which fact emphasizes the importance of this detail in technic, the hormone being more diluted in the day urine.

2. Synopsis of Series.—The cases of our series were distributed as follows:

a. Pregnant females, 61 per cent, falling into the groups shown in Table I as to duration of pregnancy.

TABLE I

LESS THAN 1 MO.	1 MO.	2 MO.	3 MO.	4 MO.	5 MO.	6 MO.	7 MO.	8 MO.	9 MO.
4.8%	11.6%	16.4%	18%	8.2%	6.6%	9.8%	8.2%	8.2%	8.2%

b. Nonpregnant females, 27 per cent, showing the following diagnoses: functional amenorrhea, 13 cases; amenorrhea following delivery, 5 cases; normal females, 4 cases; psoriasis, 2 cases; tumors, 2 cases; hydatidiform mole, 1 case.

c. Male controls, 5 per cent.

d. Discarded cases, 7 per cent, due to death of mice or insufficient clinical data.

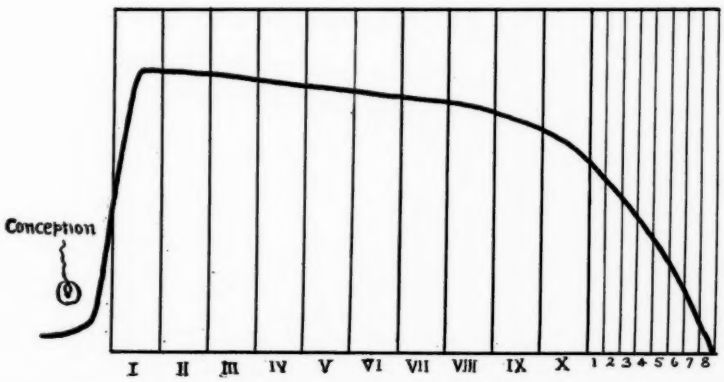


Fig. 1.—Graphic representation of anterior pituitary hormone discharge during pregnancy and puerperium. The ten lunar months are indicated by Roman numerals I to X. The eight days of the puerperium are indicated by Arabic numerals 1 to 8. Heavy solid line (———) is curve of anterior pituitary hormone discharge. (Redrawn from Aschheim and Zondek.)

3. The Earliest Possible Date at Which the Test Is Reliable.—Cases were diligently sought in which a suspicion of pregnancy in its earliest days was entertained, so that the test could be conducted, allowing the lapse of gestation to reveal its accuracy. In the following illustration redrawn from Aschheim and Zondek, a graphic representation of the discharge of pituitary hormone is shown as a solid curve. It is to be noticed that its maximal height is reached within two weeks following conception, falling off gradually throughout the period of gestation, and very abruptly through a period of ten days after delivery. It is to be borne in mind that it is the pituitary hormone, hypersecreted during pregnancy, that causes ovarian maturation and ovulation in infantile mice.

One case of fourteen days' duration gave us a distinctly positive result, which was proved by date of delivery. In the general summary Cases 35, 45, 74, were of less than one month's duration. Cases 50, 52, 56, 66, 77, 79, 83, were of

TABLE II

DETAILED SYNOPSIS OF CASES

CASE NUMBER	AGE	SEX	COLOR	PARA	DURATION OF ALLEGED PREGNANCY	NIGHT OR DAY SPECIMEN	GROSS REACTION	MICROSCOPIC REACTION	NUMBER OF MICE GROSSLY POS	NUMBER OF BLOOD PUNCTA	ENLARGEMENT OF TUBES AND UTERI	NUMBER OF INFANTILE MICE	CAUSE OF AMENORRHEA
1	22	F	W	1	108 Days	D	+	+	1	5	++	0	Normal Pregnancy
2	30	F	W	2	130 Days	D	–	?	0	0	+	0	Normal Pregnancy
3	31	F	W	4	210 Days	D	+		2	10	++	0	Normal Pregnancy
4	21	F	W	2	261 Days	D	+		2	9	++	0	Normal Pregnancy
5	21	F	W	3	283 Days	D	+		4	15	+++	0	Normal Pregnancy
6	19	F	W	2	99 Days	D	+		1	3	++	0	Normal Pregnancy
7	19	F	W	1	146 Days	D	+		1	7	++	0	Normal Pregnancy
8	19	F	W	1	164 Days	D	+		3	5	++	0	Normal Pregnancy
9	26	F	W	?	?	D	+		2	10	+++	0	? ?
10	29	F	W	2	227 Days	D	+		3	4	+++	0	Normal Pregnancy
11	34	M	W			D	–		0	0	0	5	Normal Male
12	38	F	W	7	166 Days	D	+		3	4	++	0	Normal Pregnancy
13	?	F	W	?	?	D	+		4	11	+++	0	? ?
14	36	F	W	8	193 Days	D	+		2	4	++	0	Normal Pregnancy
15	?	F	W	?	?	D	+		5	24	+++	0	Normal Pregnancy
16	24	F	W	1	5 Days After delivery of Mole	N	+	+	2	4	++	0	Hydatidiform Mole
17	19	F	W	1	187 Days	D	+		4	20	++	1	Normal Pregnancy
18	26	F	W	2	95 Days	N	+		3	12	++	0	Pregnancy—Stillborn
19	15	F	W	1	61 Days	N	0		0	0	0	0	*
20	15	F	W	1	76 Days	N	–	–	0	0	0	5	Functional Amenorrhea
21	28	F	W	2	143 Days	D	+		5	8	++	0	Normal Pregnancy
22	28	F	W			D	–		0	0	0	5	Normal Female
23	25	F	W			D	–		0	0	0	5	Normal Female
24	36	M	W			D	–		0	0	0	5	Normal Male
25	30	F	W	2	194 Days	D	+		2	2	++	0	Normal Pregnancy
26	27	F	W	1	243 Days	D	–	+	0	0	+	0	Normal Pregnancy
27	21	F	W	2	180 Days	D	+	+	3	12	++	0	Normal Pregnancy
28	32	F	W	?	?	D	+	+	1	1	+	0	? ?
29	37	F	W	1	46 Days	N	–	+	0	0	+	3	Pregnancy—Abortion
30	23	F	W	3	166 Days	D	+		5	12	+++	0	Normal Pregnancy
31	35	M	W			D	–		0	0	0	3	Normal Male
32	26	F	W	4	275 Days	D	+		1	1	++	0	Normal Pregnancy
33	36	F	W	3	246 Days	D	+		3	4	+++	0	Normal Pregnancy
34	29	F	W	1	153 Days	D	–	+	0	0	0	0	Normal Pregnancy
35	22	F	W	1	21 Days?	N	+		2	3	+	0	Pregnancy—Abortion
36	39	F	W			N	–		0	0	+	0	Psoriasis
37	28	F	W	3	198 Days	D	+		2	2	++	0	Normal Pregnancy
38	20	F	W	1	261 Days	D	–		0	0	+++	0	Normal Pregnancy
39	26	F	W	1	214 Days	D	+		1	1	++	0	Normal Pregnancy
40	21	F	W	2	225 Days	D	+		3	3	+	0	Normal Pregnancy
41	30	F	B	6	226 Days	D	–		0	0	+	0	Normal Pregnancy
42	24	F	W	2	274 Days	D	–	+	0	0	+++	0	Normal Pregnancy
43	33	F	W	?	?	D	+		1	1	++	0	Normal Pregnancy
44	21	F	W	2	232 Days	D	+	+	4	8	++	0	Normal Pregnancy
45	35	F	B	5	14 Days	D	+	+	2	2	++	0	Normal Pregnancy
46	32	F	W	2	60 Days	N	+		5	9	++	0	Pregnancy—Abortion
47	32	F	W	?	120 Days	N	+		3	6	++	0	Normal Pregnancy
48	26	F	W	1	60 Days	N	0		0	0	0	0	*
49	26	F	W	1	60 Days	N	+	+	3	6	++	0	Normal Pregnancy
50	28	F	W	2	53 Days	N	+	+	2	5	++	0	Pregnancy—Abortion
51	23	F	W	2	113 Days	N	+		1	3	++	0	Normal Pregnancy
52	32	F	W	2	49 Days	N	+		2	8	+++	0	Normal Pregnancy
53	23	F	W	1	71 Days	N	+	+	1	4	++	0	Normal Pregnancy
54	23	F	W	1	56 Days	N	–	–	0	0	+	0	Functional Amenorrhea
55	28	F	W	7	117 Days	N	+		4	11	++	0	Normal Pregnancy

*Mice died during test.

TABLE II (Continued)

CASE NUMBER	AGE	SEX	COLOR	PARA	DURATION OF ALLEGED PREGNANCY	NIGHT OR DAY SPECIMEN	GROSS REACTION	MICROSCOPIC REACTION	NUMBER OF MICE GROSSLY POS	NUMBER OF BLOOD PUNCTA	ENLARGEMENT OF TUBES AND UTERI	NUMBER OF INFANTILE MICE	CAUSE OF AMENORRHEA
56	24	F	W	1	52 Days	N	+		3	17	++	0	Pregnancy—Abortion
57	20	F	W	1	62 Days	N	+		3	6	+	0	Normal Pregnancy
58	21	F	W	2	92 Days	D	+		2	4	+	0	Normal Pregnancy
59	22	F	W	2	45 Days	N	–		0	0	+	0	Following Delivery
60	41	F	W	1	89 Days	N	–		0	0	+	0	Functional Amenorrhea
61	21	F	W	2	59 Days	N	+		5	14	+	0	Normal Pregnancy
62	21	F	W	3	240 Days	N	+		4	13	+	0	Normal Pregnancy
63	23	F	W	4	103 Days	N	+		1	1	+	0	Normal Pregnancy
64	25	F	W	2	27 Days	N	–		0	0	+	0	Functional Amenorrhea
65	28	F	W	4	63 Days	N	+		4	14	++	0	Normal Pregnancy
66	26	F	W	7	47 Days	N	+		2	9	+	0	Normal Pregnancy
67	22	F	W	4	113 Days	N	+		3	6	++	0	Normal Pregnancy
68	34	F	W	7	49 Days	N	–	–	0	0	0	5	Functional Amenorrhea
69	19	F	W	2	40 Days	N	–	–	0	0	0	5	Functional Amenorrhea
70	28	F	B	6	41 Days	N	–	–	0	0	0	5	Following Delivery
71	39	F	W	1	90 Days	N	–	–	0	0	+	1	Functional Amenorrhea
72	27	F	W	4	74 Days	N	+		4	12	+	0	Normal Pregnancy
73	27	F	W	1	?	N	–	–	0	0	+	1	Following Delivery
74	25	F	W	4	26 Days	N	+		2	12	+	0	Pregnancy—Prem. Del.
75	24	F	W	4	106 Days	N	–		0	0	++++	0	Tumor
76	35	F	W	4	99 Days	N	+		5	11	+++	0	Normal Pregnancy
77	31	F	W	7	50 Days	N	+		2	4	++	0	Normal Pregnancy
78	30	F	W	6	136 Days	N	+		1	1	+	0	Normal Pregnancy
79	27	F	W	2	58 Days	D	+		2	4	++	0	Normal Pregnancy
80	20	F	W	2	94 Days	D	+		1	1	++	0	Functional Amenorrhea
81	25	F	W	3	96 Days	N	+		2	3	+	0	Normal Pregnancy
82	29	F	B	2	24 Days	D	–		0	0	+	0	Tumor
83	29	F	W	1	42 Days	N	+		4	20	++	0	Normal Pregnancy
84	19	F	W	2	45 Days	D	–		0	0	++	0	Functional Amenorrhea
85	36	F	W	8	77 Days	N	–		0	0	+	0	Following Delivery
86	30	F	W	1	54 Days	N	–		0	0	0	0	Functional Amenorrhea
87	22	F	W	1	65 Days	N	+?		1	1	0	0	Functional Amenorrhea
88	24	F	W	1	114 Days	N	0		0	0	0	0	*
89	24	F	W	1	114 Days	N	+		1	2	++	0	Normal Pregnancy
90	35	F	W			D	–		0	0	0	2	Normal Female
91	33	F	W	1	40 Days	N	–		0	0	0	4	Functional Amenorrhea
92	40	F	W	3	46 Days	N	–		0	0	0	3	Functional Amenorrhea
93	32	F	W	6	56 Days	N	0		0	0	0	0	*
94	27	F	W	6	124 Days	N	–		0	0	0	4	Following Delivery
95	22	F	W	1	86 Days	D	–	–	0	0	+++	0	Pregnancy
96	45	F	W			N	–		0	0	+	4	Normal Female
97	36	F	W			D	–		0	0	0	5	Psoriasis
98	35	M	W			D	–		0	0	0	1	Normal Male
99	36	M	W			D	–		0	0	0	5	Normal Male
100	24	F	W	1	62 Days	N	+		2	5	++	0	Normal Pregnancy

*Mice died during test.

less than two months' duration. Cases 29, 46, 49, 53, 57, 61, 65, 72, 95, 100, were of less than three months' duration; making a total of 33 per cent of the series of less than three months' duration, the period in which clinical diagnosis is impossible or doubtful.

It will be noted that of this series of 20 cases, but one case, Case 95, was incorrect. In this instance the test was negative. The surgeon, on performing an appendectomy, stated that he had observed a pregnant uterus at the time of operation. No follow-up checking was possible.

These findings alone emphasize the value of the test in the early days or weeks of pregnancy, when clinical findings do not merit a diagnosis of the condition. The authors are of the opinion that by using 0.3 c.c. of the first morning specimen of urine positive reactions are obtainable even during the first week of gestation. This would make the test of great value in the recognition of the obscure cases of early ectopic gestation.

TECHNIC OF THE MODIFIED TEST

Apparatus and Materials Required

1. One tuberculin syringe, 2 c.c. capacity, kept in a jar of 95 per cent alcohol, for the purpose of sterilization.
2. Several 3-inch sterile funnels, for filtering urinary specimens.
3. Package of sterile filter papers to fit funnels.
4. Test tubes, 150 mm. by 30 mm., fitted with tinfoil-covered corks, sterilized in hot-air oven at 170°C. for one hour.

Fig. 2.—Photograph (magnification approximately 4X) to show internal genitalia of positive mouse (Serial Case 66), three weeks old, weight 7 gm. Fallopian tubes and ovaries show some enlargement, and are a pale pink color. Note three large corpora hemorrhagica (blood spots) projecting from the surface of the right ovary and one from the surface of the left ovary.

5. One battery jar with screen cover, and suitable bedding, etc., to house the 5 mice for each test.
6. Splinter forceps.
7. One pair straight manicure scissors.
8. One hand-magnifier, or low-power dissecting microscope.
9. One cork board, 6 inches by 12 inches by ½ inch, with pins, for dissection.
10. Immature or nymph female white mice, less than three weeks old, weighing from 5 to 8 gm.; 5 for each test.

TECHNIC

The patient is instructed to void directly into the large sterile test tube furnished by the laboratory. At least 25 c.c. of urine should be collected; cathe-

terization is unnecessary. The specimen should be secured on arising in the morning, and be immediately sent to the laboratory for refrigeration. At time of the test the urine is filtered in an aseptic manner, using the sterile funnel and sterile filter paper, and a sterile test tube for its reception. Refrigeration of the sample must be maintained throughout the duration of the test. The sterile 2 c.c. tuberculin syringe is loaded, and each of the 5 mice is injected subcutaneously with 0.3 c.c. of the urine. The injection is made at the root of the tail, on the dorsum of the animal, between the skin and muscle.

Each mouse receives 6 injections of 0.3 c.c. of filtered urine, on three successive days, as follows:

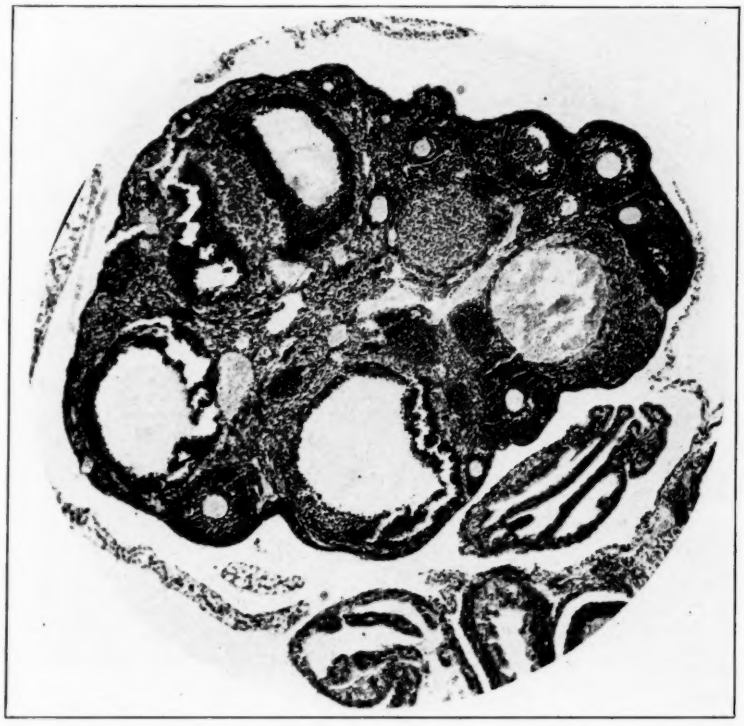


Fig. 3.—Photomicrograph (16 mm. obj., magnification approximately 100X) of section of ovary from positive mouse (Serial Case 49). Note enlarged mature graafian follicles, some showing ova. Also note two corpora lutea, one corpus hemorrhagicum in the upper right hand portion of the section.

Same day urine is received, injections at 1 P.M. and 5 P.M.

Second day, injections at 10 A.M., 1 P.M., and 5 P.M.

Third day, injections at 10 A.M.

On the afternoon of the fifth day, fifty-two hours after the last injection, the mice are killed by passing illuminating gas into battery jars by means of a hose, the top of the jar being covered by cardboard.

Dissecting is accomplished by means of the splinter forceps and manicure scissors. Good illumination and the magnifying glass will facilitate the procedure. The incision is made in the median line, the intestines being pushed upwards to expose the uterus and tubes. The ovaries will be found in the vicinity of the lower pole of each kidney. They can be drawn down by means

of the splinter forceps, and studied carefully under a dissecting microscope or magnifier, without removal. Positive tests depend upon the finding of one or more black spots from ½ to 1 mm. in diameter, projecting as spheric nodules

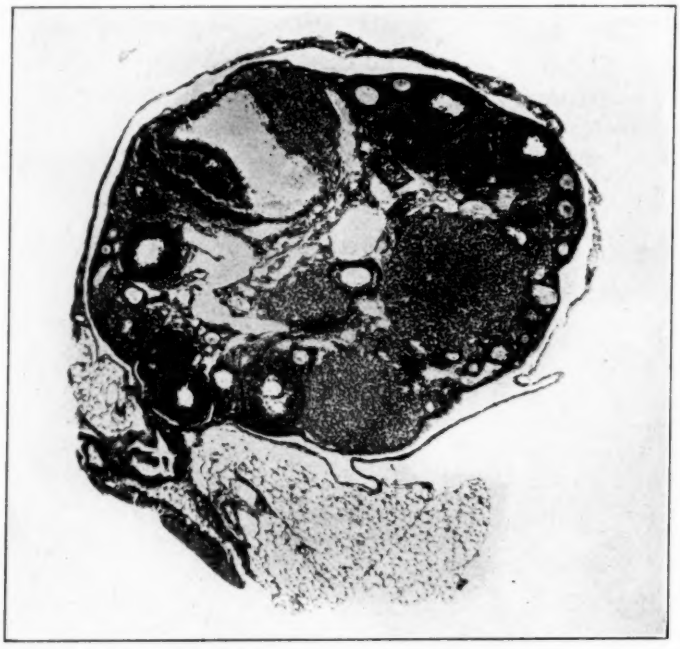


Fig. 4.—Photomicrograph (16 mm. obj., magnification approximately 100X) of section of right ovary, mouse No. 3 (Serial Case 16). Note numerous mature graafian follicles, many with ova. In the upper left hand portion is seen a developing corpus luteum with corpus hemorrhagicum, and also two well-marked corpora lutea in lower right portion.

Fig. 5.—Photograph (magnification approximately 4X) showing internal genitalia of mouse, three weeks old, weight 7 gm. Note the small size of uterus, tubes, and ovaries; also absence of follicular development and corpora hemorrhagica (blood spots).

from the ovarian surface. These are the "blut punkten" (corpora hemorrhagica) of Aschheim and Zondek. (See Fig. 2.) In the absence of these, the test may

still be positive, but requires histologic study of the ovum for revelation of the corpus luteum with or without hemorrhage. (See Figs. 3 and 4.) If the pregnancy test be negative, no gross nor histologic corpora hemorrhagica nor corpora lutea are detectable. (See Figs. 5 and 6.)

Fig. 6.—Photomicrograph (16 mm. obj., magnification approximately 100X) of section from ovary of negative mouse (Serial Case 73). Observe numerous immature, small, and one large, graafian follicle. No corpora lutea nor corpora hemorrhagica are present.

ADVANTAGES OF THE MODIFIED TECHNIC OVER THE ORIGINAL

The use of 0.3 c.c. of the patient's filtered urine given to each of the 5 mice obviates the necessity of labelling or isolating each mouse separately according to dosage. The maximum dose, 0.3 c.c., gives a clearer-cut reaction even when the day urine is used.

CONCLUSIONS

The use of male urine seems to have no effect upon the mice; the uterus, tubes, and ovaries remaining infantile.

In case of hydatidiform mole a positive reaction was obtained.[2]

Tubal and ovarian maturation in the injected mouse is proportionate, practically in all cases, to the duration of the pregnancy in the woman from whom the urine was obtained.

Unfortunately no cases of pituitary hyperfunction were included in our series.

The possibility of false positives in cases of hyperfunction must be considered, but these cases are easily excluded by their symptom complex.

The number of previous pregnancies in each case tested seems to have no influence whatsoever on the reaction.

Color, race, and age are without bearing.

It is evident in normal female controls, i.e., nonpregnant women with regular menses, that the mice remain infantile after injection; whereas in nonpregnant females suffering with functional amenorrhea, there is a tendency for the uterus and tubes in the mice to be slightly enlarged, and for the hymen to disappear, without however changes in the ovary, the latter being the significant finding in a positive test.

The chief disadvantage of the test in the modern clinical laboratory is the difficulty of having on hand immature female mice, less than three weeks old, and weighing not more than 8 grams. A minimum of 15 female breeder mice, and 5 mature males is necessary, but mating is not always controlled, due to seasonal influence.

In certain instances the test cannot be performed due to toxicity of the urine causing death of the mice.

The number of injections (30), over a period of three days is extremely laborious.

The recognition of sex in nymph mice may be difficult to the average pathologist.

Dissection requires some skill and anatomic knowledge due to the miniature nature of the organs.

Up to date we believe that the Aschheim-Zondek test as herein modified, is the most accurate method for establishing a diagnosis of pregnancy. This method has given the earliest possible diagnosis of pregnancy, and the clinician will see clearly that the test would be of help in the diagnosis of ectopic gestation.

The authors wish to express their appreciation to Dr. Harriet L. Hartley, Bureau of Child Hygiene, Philadelphia, and to the various members of the staffs of Temple University and Greatheart Hospitals, for their cooperation in furnishing clinical material for this work.

REFERENCES

1. Aschheim, S., and Zondek, Bernhard: Die Schwangerschaftsdiagnose aus dem Harn Durch Nachweis des Hypophysenvorderlappenhormons, Klin. Wchnschr. **7**: 1404, 1928.

Aschheim, S., and Zondek, Bernhard: Schwangerschaftsdiagnose aus dem Harn (Durch Hormonnachweis), Klin. Wchnschr. **7**: 8, 1928.

2. Fanz, J. I., and Gault, E. S.: Hydatidiform Mole as a Cause of Positive Reaction in the Aschheim-Zondek Pregnancy Test, J. LAB. & CLIN. MED. **16**: 27, 1930.

A METHOD FOR THE COLORIMETRIC DETERMINATION OF ARSENIC*

By Guy E. Youngburg, Ph.G., Ph.D., and Jason E. Farber, Buffalo, N. Y.

THIS paper presents a rapid but accurate method for the determination of arsenic in biologic materials, organic arsenicals, etc. Although many methods and modifications of them have been proposed in the past, and especially in the last several years, no one has proved to be entirely satisfactory. Evidently improvements are still sought. A comprehensive review of the literature up to 1927 is found in the papers of Minot[1] and Kleinmann and Pangritz.[2] Since 1927, a number of methods of different types have been proposed.[3, 4, 5, 6, 7†]

We have found that the direct determination of arsenic colorimetrically, after oxidizing its sulphide,‡ is very satisfactory. The organic matter containing arsenic, is oxidized with sulphuric acid, nitric acid and perhydrol and the arsenic is precipitated as sulphide. This is then readily oxidized with sulphuric acid and perhydrol and a blue color is developed by the addition of molybdate and stannous chloride.

Stannous chloride reduction of arsenomolybdic acid gives much more color than other reducing agents which have been used up to the present time. This reducing agent is being used principally for phosphorus determinations, [8, 9, 10] but is applicable to arsenic.[11, 12, 13]

Several of the special reagents employed in the following method are those used in this laboratory for phosphorus determinations and the colorimetric procedure is essentially the same. For the sake of convenience we redescribe several of the reagents and a part of the procedure which has been published elsewhere for phosphorus.[8]

METHOD

Special reagents required:

1. Ten Normal Sulphuric Acid.—450 c.c. of concentrated sulphuric acid,§ c.p. are added to 1100 c.c. of water. This solution is titrated and diluted to make 10 normal.

2. Molybdate-Sulphuric Acid Mixture.—

Solution A. Mix 50 c.c. of 7.5 per cent sodium molybdate ($Na_2MoO_4 \cdot 2H_20$) c.p., P-free, and 50 c.c. of 10 N sulphuric acid.

Solution B. Mix 50 c.c. of 7.5 per cent sodium molybdate, c.p., P-free, 25 c.c. of water and 25 c.c. of 10 N sulphuric acid.

---

*From the Department of Biological Chemistry, University of Buffalo, Medical School. Received for publication, July 30, 1931.

†Although we use a somewhat similar oxidation and sulphide precipitation to that of Fellenberg,[6] the subsequent process is entirely different. Also the method of Poljakow and Kolokolow[5] bears no similarity to ours except that they finally use a stannous chloride reduction of arsenomolybdate (after Feigl). Their concentrations differ widely from ours since they are not adjusted to give the maximum color possible.

‡Sulphide precipitation for the quantitative determination of elements in small amounts is not unusual.[6, 14, 15, 16]

§While arsenic-free brands of sulphuric acid can be purchased, the c. p. acid used for general laboratory purposes is usually practically arsenic-free. Blanks must be run, however.

3. Stannous Chloride Solutions.—(Stock and dilute solutions according to Kuttner and Cohen.)

Dissolve 10 grams of stannous chloride, c.p. (preferably Kahlbaum's) in 25 c.c. of concentrated hydrochloric acid, c.p. Store in a brown, glass-stoppered bottle.

Dilute 0.5 c.c. of the above stock solution to 100 c.c. with water. This solution is safe to use for a week or until a turbidity forms. After that a new dilution should be made.

4. Standard Arsenic Solution.—Dissolve 0.1533 grams of pure arsenic pentoxide in 50 c.c. of dilute sodium hydroxide solution (P-free). Neutralize with sulphuric acid and make up to 100 c.c. with water. 1 c.c. = 1 mg. of arsenic.

From the above, dilutions are made so that 1 c.c. = 0.1 mg. and 1 c.c. = 0.01 mg. of arsenic. Preserve with chloroform. These solutions keep for at least six months and perhaps indefinitely.

5. Perhydrol.—Thirty per cent hydrogen peroxide. Merck Blue Label Superoxol is satisfactory since it contains inappreciable amounts of phosphorus or arsenic. Keep in the refrigerator after daily use.

Procedure:

(The following description is for body tissues. For other materials analyzed, the approximate amounts to be taken must be estimated.)

Fifty grams* of tissue are cut into small pieces, placed in a 500 c.c. Kjeldahl flask and covered with concentrated nitric acid. To it is added 10 c.c. of concentrated sulphuric acid, several silica† or other pebbles (to prevent bumping) and several drops of caprylic alcohol (to prevent foaming). The flask is then heated, at first gently, then vigorously until fumes from the sulphuric acid appear. Oxidation is completed by using more nitric acid and finally perhydrol (by drops; total about 15 c.c.). The contents of the flask are washed into a 50 c.c. Erlenmeyer flask, made up to a volume of 20 c.c. with water and cooled to room temperature. Hydrogen sulphide is passed into the cold solution for about five minutes and the flask is then heated to 70-90° while the hydrogen sulphide continues to pass in slowly for an additional five to ten minutes. The flask is then stoppered and set aside overnight.‡ The supernatant liquid is then decanted, the precipitate is centrifuged and washed by centrifuging with 4 to 6 normal sulphuric or hydrochloric acid 3 or 4 times, except that the last washing is done with water.

The precipitate is then transferred to a 150 × 20 mm. Pyrex test tube graduated at 10 c.c. Five-tenths c.c. of 10 normal sulphuric acid and a silica pebble are added, the tube is placed slanting partly over an electric hot plate or small flame and heated at a boil until the water is evaporated and the acid begins to boil gently. The tube is then temporarily removed and after allowing it to cool for about thirty seconds, 1 or 2 drops of perhydrol are added. The tube is replaced and the heating continued until oxidation is complete, more perhydrol

*Often less tissue may be taken, depending on the amount of arsenic present or the amount of tissue available.

†Broken silica-ware can usually be obtained from the organic chemistry laboratory.

‡The sulphide is first in a very finely divided state and is slow in settling out. If necessary, the solution can be centrifuged within an hour after precipitation.

being added if necessary. Any possible excess of perhydrol must be removed by continued boiling for five minutes, otherwise the color development will be retarded.

The tube is then placed in a rack, allowed to cool, water is added to the 10 c.c. mark and the contents are mixed. An aliquot (e.g., 5 c.c.) is removed and kept for possible later use.

To the remaining aliquot is added enough 10 normal sulphuric acid to make a total of 0.5 c.c. of that acid and 2 c.c. of molybdate-sulphuric acid solution B.

A standard arsenic tube is then prepared by transferring 5 c.c. (0.05 mg. As) of standard arsenic solution in a similar tube and adding 2 c.c. of molybdate-sulphuric acid solution A.

To both tubes are then added 1 c.c. of dilute stannous chloride solution and water to the 10 c.c. mark and the contents are mixed without delay. It is best to complete each tube independently.

The color is read in a colorimeter after one minute.

If the color of the unknown greatly exceeds that of the standard, dilution may be made, but for most accurate work a smaller aliquot must be taken for the color development.

Calculation:

When a 5 c.c. aliquot is taken and the standard is set at 20 mm.: 20/ reading of the unknown $\times$ 0.2 = mg. arsenic in 100 grams of tissue.

DISCUSSION

To oxidize organic matter, preliminary to the precipitation of the arsenic as sulphide, we use a modification of the Sanger method, employing sulphuric and nitric acids and perhydrol. While this combustion is time-consuming, it compares favorably with other methods in that regard, and on the whole is the most satisfactory. Fifty grams of beef liver, for example, can be oxidized in one and five-tenths hours.

It is of course impossible to designate the exact procedure for all determinations because of the variety of materials analyzed. Blood is treated like solid tissues. Urine and beverages require concentration to small volume by evaporation as a preliminary process. Organic arsenicals are easily oxidized by sulphuric acid and perhydrol, omitting the nitric acid. Catalytic agents such as ferric chloride, copper sulphate and permanganate cannot be used because they interfere with the sulphide precipitation. Neither is it advisable to use potassium chlorate and hydrochloric acid in the oxidation on account of the volatility of arsenic trichloride.

The conditions for the precipitation of arsenic as sulphide were closely studied. We desire to precipitate in sulphuric acid solution, if possible, because the digestion is carried out in this acid. Although hydrochloric acid has customarily been used in qualitative procedure, we find that sulphuric acid works equally well. Table I shows how completely the sulphide is precipitated in small quantities and under varying degrees of acidity. It is seen that 0.01 mg. of arsenic can be precipitated with a good degree of accuracy from a volume of 20 c.c.

The rate of precipitation varies directly with the concentration of H ions and the temperature.[17] However, too much acid, on account of its high gravity, prevents sulphide from being thrown down when centrifuging. The optimum acidity lies between 25 and 50 volumes per cent.

Some hydrogen sulphide is always reduced in this process, and some free sulphur thus liberated, but this is desirable because it entrains the sulphide and facilitates the handling of it. We do not believe that so small a quantity of arsenic as 0.01 mg. could otherwise be recovered.

Antimony, which is rarely present, is prevented from precipitating with the arsenic when 40 volumes per cent of acid is used in the precipitation of the sulphide.[18] It is later eliminated in the washings along with the phosphate.

An intense blue color is developed by arsenic under the conditions described in this method. It is 0.4 as intense as the color produced by phosphorus with the same reagents. Fig. 1 shows that the rate of fading of the blue color is very slow. These data were obtained by preparing a standard arsenic tube and then comparing the color with standard arsenic tubes freshly prepared at the times indicated. The temperature effect on the color development is so small that it can be neglected.

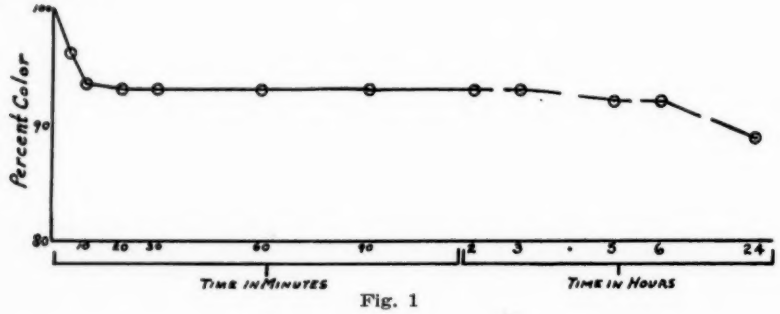


Fig. 1

TABLE I

RECOVERY OF ARSENIC FROM PURE AQUEOUS SOLUTIONS OF ARSENIC SALTS, WHEN VARYING THE ACIDITY FOR THE SULPHIDE PRECIPITATION

NO.	SULPHURIC ACID VOL. PER CENT	ARSENIC ADDED MG.	ARSENIC RECOVERED MG.	RECOVERY PER CENT
1	2.4	1.00	0.997	99.7
2	4.8	1.00	0.997	99.7
3	7.2	1.00	1.003	100.3
4	9.5	1.00	0.985	98.5
5	14.3	1.00	0.995	99.5
6	19.1	1.00	1.040	104.0
7	23.9	1.00	0.985	98.5
8	28.6	1.00	1.000	100.0
9	33.4	1.00	1.000	100.0
10	47.5	1.00	1.020	102.0
11	33.4	0.01	0.013	113.0
12	33.4	0.01	0.010	100.0
13	33.4	0.03	0.028	96.2
14	33.4	0.03	0.031	102.0
15	28.6	0.05	0.049	98.7
16	28.6	0.10	0.096	96.0
17	28.6	0.10	0.097	97.0
18	28.6	0.50	0.499	99.8
19	28.6	1.00	0.995	99.5
20	28.6	1.00	0.997	99.7

TABLE II

RECOVERY OF ARSENIC FROM LIVER

NO.	ARSENIC ADDED MG.	RECOVERED MG.	RECOVERY PER CENT
	From 3 Grams of Liver		
1	0.00	0.001	—
2	0.00	0.003	—
3	0.05	0.048	96.0
4	0.05	0.049	98.0
5	0.10	0.098	98.0
6	0.10	0.096	96.0
7	0.50	0.491	98.2
8	0.50	0.488	97.6
9	0.75	0.716	95.5
10	1.00	0.967	96.7
11	1.00	0.943	94.3
12	3.00	2.901	96.7
13	3.00	2.840	94.0
14	5.00	4.800	96.0
15	5.00	4.680	93.6
	From 50 Grams of Liver		
16	0.10	0.095	95.0
17	1.00	0.971	97.1
18	1.00	0.954	95.4
19	1.00	0.964	96.4

Table II shows the accuracy of the method in the recovery of arsenic from a tissue. With the smaller amounts of tissue the recovery range is shown to be between 93 and 98 per cent when 5 to 0.05 mg. of arsenic is present. With 50 grams of tissue (which may often be taken in practice) the recovery is the same.

Using the standard arsenic solution (not involving the sulphide precipitation) one part of arsenic in 10,000,000 parts can be detected. This is not as sensitive as the Marsh test, but equals that of the Gutzeit method. It is about 250 times as sensitive as the Reinsch test.

SUMMARY

Arsenic is determined by precipitating as sulphide from the oxidized material. The sulphide is then oxidized and determined colorimetrically by the addition of molybdate and stannous chloride.

After the oxidation of organic matter, the method is simple, accurate, and very sensitive.

It is applicable to almost any arsenic determination but was developed primarily for biologic and toxicologic work.

REFERENCES

1. Minot, A. S.: Distribution of Arsenic in Tumor-bearing Mice, J. Cancer Research **10**: 293, 1926.
2. Kleinmann, H. S., and Pangritz, F.: Eine nephelometrische Methode zur Bestimmung kleinen Arsenmengen, Biochem. Ztschr. **185**: 14 and 44, 1927.
3. Cislak, F. E., and Hamilton, C. S.: A Method of Determining the Arsenic Content of Organic Arsenicals, J. Am. Chem. Soc. **52**: 638, 1930.
4. Fink, D. E.: Modified Electro-Gutzeit Apparatus for Quantitative Estimation of Minute Quantities of Arsenic in Insect Tissue, J. Biol. Chem. **72**: 737, 1927.
5. Poljakow, A., and Kolokolow, N.: Ein Verfahren für die kolorimetrische quantitative Arsenbestimmung, Biochem. Ztschr. **213**: 375, 1929.

6. Fallenberg, Th. von: Eine rasch ausführbare Mikroarsenbestimmungsmethode für organische Stoffe, Biochem. Ztschr. **218**: 283, 1930.
7. Maechling, E. H., and Flinn, F. B.: Colorimetric Determination of Small Amounts of Arsenic in Biologic Material, J. LAB. & CLIN. MED. **15**: 779, 1930.
8. Youngburg, Guy E., and Youngburg, Mamie V.: Phosphorus Metabolism. I. A System of Blood Phosphorus Analysis, J. LAB. & CLIN. MED. **16**: 158, 1930.
9. Levene, P., and Raymond, A. T.: Hexosediphosphate, J. Biol. Chem. **80**: 633, 1928.
10. McKay, C. M.: Phosphorus Distribution, Sugar and Hemoglobin in the Blood of Fish, Eels and Turtles, J. Biol. Chem. **90**: 497, 1931.
11. Truog, E., and Meyer, A. H.: Improvement in the Denige's Colorimetric Method for Phosphorus and Arsenic, J. Ind. Eng. Chem., Anal. Ed. **1**: 136, 1929.
12. Atkins, W. R. G., and Wilson, E. G.: Colorimetric Method of Estimation of Minute Amounts of Silicon, Phosphorus and Arsenic, Biochem. J. **20**: 1223, 1926.
13. Feigl, F., and Neuber, F.: Beitrage zum Nachweiss der Elemente der H_2S-Gruppe mit besonderer Berüchsichtigen Tupfelanalyse, Ztschr. f. anal. Chem. **62**: 369, 1923.
14. Fairhall, L. T., and Prodan, L.: Colorimetric Determination of Minute Amounts of Cadmium, J. Am. Chem. Soc. **53**: 1321, 1931.
15. Fairhall, L. T., and Richardson, J. R.: The Nephelometric Analysis of Zinc, J. Am. Chem. Soc. **52**: 938, 1930.
16. Aub, J. C., Fairhall, L. T., Minot, A. S., and Reznikoff, P.: Lead Poisoning, Medicine IV **1**: 1925.
17. Noyes, A. A.: Qual. Chem. Anal. New York. The Macmillan Co., 1915, p. 62, note 2.
18. Neher, F.: Eine rasche Methode zur vollständigen Fällung des Arsen als Pentasulfid und zur Trennung desselben von Bismuth, Bei, Antimon und ähnlichen Metallen, Z. anal. Chem. **32**: 45, 1893.

A NEW PROCEDURE FOR LIGATING THE PYLORUS IN ABSORPTION EXPERIMENTS*

By Stephen J. Maddock, Boston, Mass.

A SURVEY of the methods which have been reported for studying absorption from the stomach reveals that all of them may be included under two general headings: (A) acute experiments using general anesthesia or, (B) a series of experiments on animals provided with alimentary fistulas.

Employing the first of these general procedures a number of investigators have anesthetized animals, opened the abdomen and ligated the pylorus. Then they have introduced into the stomach the substance to be studied and after a definite interval have killed the animal and analyzed the material recovered from the stomach at that time. By means of such processes much useful information has been collected. This we shall not attempt to summarize, but merely point out that in connection with such experiments the question always has arisen as to whether or not absorption proceeded at a normal rate while the animals were under anesthesia.

The second of the above mentioned general methods has involved the preparation of fistulas giving direct access either to the stomach or duodenum, or both. Many of the early investigators used duodenal fistulas, and after blocking the distal end of the duodenum with a rubber balloon, caught in a container the material which flowed out from the pylorus. Gastric fistulas were used also, and the pylorus blocked by a balloon inserted from above. In both instances the difficulty of obtaining quantitative recovery of the unabsorbed material is obvious. A number of years ago London (1908, 1913) described and used, apparently quite successfully, a very ingenious and elaborate double fistula technic for overcoming this last difficulty. Quite recently, however, a new and hitherto unsuspected element of uncertainty has appeared. Gamble and McIver (1925, 1928) have brought to light the deleterious effect of complete loss of gastric or duodenal secretions. If there was even a slight but continuous loss of body fluids in the animals with fistulas, it is possible that some abnormality might have been introduced into their response. Since the various investigators who have used this device made no specific mention of leakage from fistulas it has not been possible to judge either the extent or the possible significance of this factor.

Consideration of the difficulties and uncertainties of the methods previously used led to the design of an experimental procedure which avoided both the complications of ether anesthesia and the effect of fistulous openings into the alimentary tract. This method served very satisfactorily in the investigation on carbohydrate absorption which occasioned its development. The possible usefulness of it to those who might wish to carry out similar studies upon other substances under conditions so nearly physiologic prompt us to describe the

*From the Biochemical Laboratory of Harvard Medical School.
Received for publication, July 21, 1931.

method in this place. That it is also applicable to the problem of following the early changes in body fluids which follow pyloric obstruction will be evident.

EXPERIMENTAL

The Preliminary Operation.—All the experiments in this series were performed upon healthy adult dogs. Under ether anesthesia and with aseptic technic a midline incision 12 to 15 cm. long extending downward from the tip of the xiphoid was made. The properitoneal fat was separated and the stomach exposed. The hepatoduodenal ligament was divided sufficiently to allow the pylorus to be brought into the wound. After a little dissection it was possible to pass a flat tape around the pylorus without injury to any of the vessels of the omentum. The ends of the tape were then inserted through a special glass cylinder about 3 to 4 cm. in length and of 1.7 cm. internal diameter (Fig. 1). These cylinders were open at both ends and the edges were provided with broad

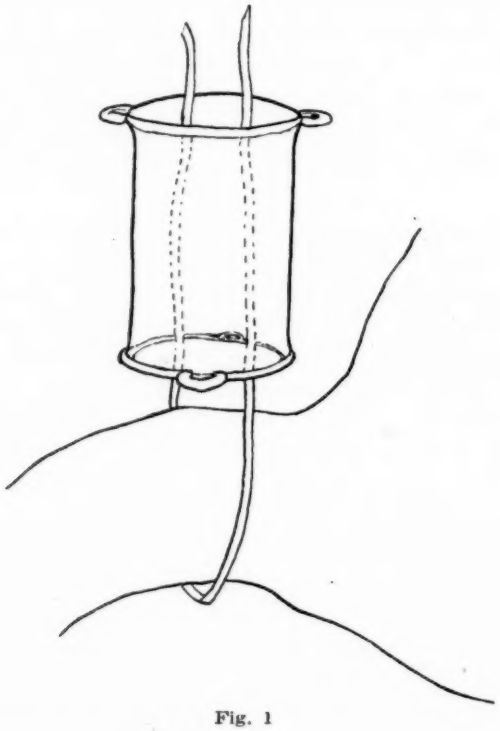


Fig. 1

flanges each of which carried two eyelets in the glass. These eyelets were useful in anchoring the glass cylinder loosely to the pyloric region of the stomach and to the incised edges of the linea alba. Care was taken to insure that the relations of the pylorus were undisturbed and that the glass tubing did not fixate the stomach and thus cause kinking or obstruction. The tape was then anchored loosely to the subcutaneous tissues with its ends protruding from the glass cylinder. The incision was closed with interrupted sutures of silk so that the outer end of the glass tubing was located near the upper angle of the wound. Animals prepared in this way recovered quickly and their appetites and food

consumption promptly became normal. Usually the animals were subjected to absorption experiments within three or four days. However, in several instances longer periods elapsed without any noticeable effect upon the results obtained. These animals should not be kept more than six or seven days, since the tape tends to disintegrate, and after that period it may break when it is pulled taut.

The Absorption Experiment.—After a preliminary fast of eighteen to twenty-four hours a control sample of blood was drawn from the animal. Then by the severance of the stitches holding the skin the ends of the tape and the edge of the glass cylinder were exposed. The snare about the pylorus was slowly and gently drawn tight and fixed by a clamp resting against the edge of the glass tube. A stomach tube was then introduced by mouth into the esophagus and a solution of the substance being studied was allowed to flow into the stomach. The dogs never showed any signs of discomfort and no retching or vomiting occurred for at least two hours. They were allowed to remain on the table or to move about the room. The animals in this series were sacrificed at the end of two hours by the intravenous administration of amytal. In each instance the complete closure of the pylorus was demonstrated at autopsy.

Results.—In Tables I and II will be found data which illustrate the applicability of the method for its purpose. Observations upon the blood sugar content of the peripheral veins of dogs before, and just after, the manipulations necessary to close the pyloric snare are contained in Table I. It will be evident that the procedures employed were without hyperglycemic effect. The Folin-Wu method (1919) was employed in making the blood sugar measurements.

TABLE I

EFFECT OF CLOSURE OF PYLORIC SNARE UPON THE BLOOD SUGAR

EXPERIMENT NUMBER	BEFORE	AFTER
	MG. PER CENT	MG. PER CENT
1	78*	78
2	93	100
3	77	84
4	80	73
5	77	70
6	100	81
Averages	84	81

*All samples drawn from peripheral veins.

TABLE II

BLOOD SUGAR CHANGES DURING GLUCOSE TOLERANCE TESTS

TIME	PYLORIC SNARE (A) OPEN	PYLORIC SNARE (B) CLOSED
MINUTES	MG. PER CENT	MG. PER CENT
Control	72	78
25.5 gm. Glucose in 200 c.c. Water Introduced Into Stomach.		
15	93	83
30	105	85
60	149	82
90	165	87

Weight of dog: 8.5 kilos.
Pyloric snare introduced: Nov. 11, 1928.
Experiment (A) Nov. 17, 1928.
Experiment (B) Nov. 20, 1928.

Table II records the data of some blood sugar studies made upon a dog after a pyloric snare had been put in place as described above. In experiment (A) the snare had been in position for several days, but had not been closed. In experiment (B) the snare was closed after the control blood sample had been taken but before the sugar was introduced.

In the experiment in which the snare was in place, but open, it may be noted that glucose produced a normal rise in the sugar content of the peripheral blood. On the other hand when the experiment was repeated a few days later with the snare closed, the sugar fluctuated only slightly and an increase in excess of the uncertainties of sampling and measurement was not obtained.

SUMMARY

A procedure is described by which the pylorus can be obstructed when desired without the complication of general anesthesia. A snare, consisting of a glass tube and a flat tape encircling the pylorus, is inserted at a preliminary operation. This is allowed to remain in place for several days while the animal recovers. Then by means of this device the pylorus can be closed readily from the exterior just before an experiment is to be undertaken. A series of blood sugar values is reported which show the application of the method to the study of the absorption of glucose from the stomach.

This procedure has been developed during the course of an investigation carried on in collaboration with Professor Harry C. Trimble.

REFERENCES

Folin, O., and Wu, H.: A System of Blood Analysis, J. Biol Chem. **38**: 81, 1919.

Gamble, J. L., and McIver, M. A.: Effects of Pyloric Obstruction in Rabbits, J. Clin. Investigation **1**: 531, 1925.

Gamble, J. L., and McIver, M. A.: Body Fluid Changes Due to Continued Loss of the External Secretion of the Pancreas, J. Exper. Med. **48**: 859, 1928.

London, E. S.: Zum Chemismus der Verdauung im Thierischen Körper, Ztschr. Physiol. Chem. **53**: 246, 1907.

London, E. S., and Tschekunow, J. S.: Weiterer Unterschungen über die Verdauung und Resorption unter normalen und pathologischen Verhaltnissen, Ztschr. Physiol. Chem. **87**: 314, 1913.

A SIMPLE METHOD FOR THE ANALYSIS OF PROTEIN IN MILK*

By Charles F. Crowley, A.M., Ph.C., M.D., LL.D., Omaha, Neb.

THOSE far removed from well-equipped laboratories may have occasion to require the protein content (casein, lactoglobulin, and lactalbumin) of milk, when facilities for the Kjeldahl method are not available. Reliable results may be quickly and easily obtained by the following procedure, using a centrifuge and 15 c.c. tubes.

Centrifuge 15 c.c. of milk and remove the cream layer. It may be thrown off with a sudden jerk over the sink or, with a pipette, the skimmed milk can be drawn from below. Dilute 5 c.c. of this skimmed milk with 20 c.c. of water and shake well. Place 5 c.c. of the dilution in a Purdy 15 c.c. centrifuge tube, add 5 c.c. of water and 5 c.c. of Esbach's picric acid solution, then shake well.

ESBACH'S SOLUTION

Picric acid	1 gm.
Citric acid	2 gm.
Water	100 c.c.

Place tubes in the centrifuge and run centrifuge for just three minutes. The number of cubic centimeters of precipitate in the conical bottom of the tube multiplied by 6 will approximate the amount of protein in the milk in percentage.

The centrifuge used in our experiments has a diameter of 15 inches, and a speed of 1,300 revolutions per minute. Milk containing, by the Kjeldahl method, 3.5 per cent of protein gave a volume precipitate of 0.6 c.c. in the conical end of the tube. This multiplied by 6 gives 3.6 per cent of protein.

Whole milk cannot be used in the above method until the fat is removed, as the amount of fat swells the volume of the precipitate and is sometimes so large, as compared to the protein, that a floating instead of a sinking coagulum is formed.

Comparison Table

KJELDAHL METHOD		CENTRIFUGE METHOD
3.50	Cow's Milk	3.60
3.43	Cow's Milk	3.30
3.31	Cow's Milk	3.15
3.32	Cow's Milk	3.15
3.50	Cow's Milk	3.30
2.27	Cow's Milk	2.40
2.10	Cow's Milk	2.40
3.06	Goat's Milk	3.00
3.06	Goat's Milk	3.00

With a centrifuge having a less diameter and a less number of revolutions per minute, a smaller constant or multiplier than 6 would be used as the volume of the precipitate would be larger. The three factors controlling the volume of

*From the Department of Public Affairs, Omaha.
Received for publication, December 19, 1930.

the precipitate are diameter of the centrifuge, speed, and time. These can be constant for anyone carrying on the above method.

The merit of this method is in its availability irrespective of diameter, speed, and time, inasmuch as one can determine the volume of the precipitate for a sample of normal milk, with his particular centrifuge, with its particular diameter and speed and specific time of three minutes. Any sample having a less volume will of course be below normal, while greater volumes are above normal. This is true even when the tubes are not very correctly graduated as is sometimes the case.

Should other components of milk be of interest the following details can be followed:

Take the specific gravity with the urinometer spindle. Variation is usually between 1.025 and 1.035. Always make correction for temperature on the basis of one gravity degree (third decimal place) for each seven Fahrenheit degrees above or below 60° F. as the spindles are standardized for 60° F. Add if above, and subtract if below.

Determine the fat content by the Babcock method. Use 5 c.c. of milk, add 5 c.c. of sulphuric acid (sp. gr. 1.80), one c.c. at a time, shake well after each addition. Centrifuge for three minutes, add hot water to force the fat up into the measuring neck of the tube, centrifuge one minute and read the amount of fat.

Total solids can be found by Richmond's formula: $1.2 \times$ Fat $+ 0.25 \times$ Gravity (second and third figures) $+ 0.14 =$ Total solids. Example: Sp. gr. $= 1.032$ and Fat $= 3.8$ per cent. $1.2 \times 3.8 + 0.25 \times 32 + 0.14 = 12.70$ Total solids.

The total solids can also be determined by weighing a dish with 10 gm. of sand and a stirring rod, adding 5 gm. of milk, placing in an oven at 100° C. and evaporating to constant weight. Increased weight multiplied by 20 gives the percentage of solids.

The ash may be determined by evaporating 5 gm. of milk in a small crucible to dryness and then incinerating. The increased weight of the crucible multiplied by 20 will be the percentage of ash, or inorganic solids.

It is quite evident that a sugar determination is not necessary as the sum of the protein, fat, and ash subtracted from the total solids would equal the lactose.

Milk Sugar.—Lactose may be determined by taking 5 gm. of milk and adding 40 c.c. of water and 5 c.c. of glacial acetic acid. Boil, cool, and make volume up to 50 c.c. Filter this mixture into a burette and from it run the solution slowly into boiling Purdy's ammoniacal copper solution, until the blue color just disappears. Use 35 c.c. of this solution.

PURDY'S AMMONIACAL COPPER SOLUTION

Copper sulphate	4.752 gm.
Caustic Potash (KOH)	23.500 gm.
Ammonium Hydroxide (sp. gr. 0.9)	350.000 c.c.
Glycerol $(C_3H_5OH)_3$	38.000 c.c.
Water to	1000.000 c.c.

Since 35 c.c. of this solution is reduced by 0.02 gm. of glucose or by 0.03 gm. of lactose, it is only necessary to divide the number of cubic centimeters required to decolorize the 35 c.c. of Purdy's solution into 0.03 and multiply by 100 to obtain the percentage of lactose in the dilution and by 10 again, as the 5 gm. of milk were diluted to 50 c.c. Of course Benedict's solution can be substituted for Purdy's, but remember that the 25 c.c. of Benedict's solution is reduced by 0.05 gm. of glucose and 0.067 gm. of lactose.

Where a polariscope is available such as Ultzman's, measure out 25 c.c. of milk, add 0.5 c.c. acid mercuric nitrate solution (made by dissolving mercury in twice its weight of nitric acid sp. gr. 1.42 and diluting with an equal volume of water) and 2 c.c. of water. Shake, let stand five minutes, filter and polarize. Multiply reading by 1.1. The specific rotating power of glucose and lactose are about the same at 20° C., viz., 52.5.

Inasmuch as the foregoing directions are for rapid work and the results are approximations, it makes little difference whether 5 c.c. of milk are used where 5 gm. are indicated, and vice versa.

A PERMANENT NITROPRUSSID SOLUTION FOR ACETONE TESTS*

By Robert M. Hill, Ph.D., Denver, Colo.

IN URINALYSIS, acetone tests which make use of nitroprussid can have no quantitative significance and have rather questionable comparative value unless the nitroprussid solution be of the same strength for each determination. A water solution of nitroprussid decomposes so rapidly in the light as to make the quantitative use of such a solution impossible. Semiquantitative methods based upon the nitroprussid reaction have lacked much in convenience because of the necessity of preparing the reagent each day with quantitative care. Because this procedure was irksome and because of the loss of time involved, a study of the problem of stabilizing the nitroprussid solution was undertaken in this laboratory about two years ago. After many failures, one successful method was found and it alone will be discussed.

It was thought that the free acid might be more stable in solution than the salt. Two per cent solutions of sodium nitroprussid were made in sulphuric acid† of strengths ranging from 1 to 5 per cent. Samples of these were put in clear glass and in brown glass bottles, placed on the laboratory shelf and examined from time to time. Deterioration was measured by the appearance of Prussian blue and by color comparison with a freshly made solution. These solutions have been standing now for eighteen months, portions being used from time to time for acetone tests. Only the solution in one per cent sulphuric acid in the clear glass bottle has shown any deterioration. A trace of blue color appeared in the bottom of this bottle during the eleventh month. The nitroprussid in one per cent acid in the dark glass bottle is still as good as when prepared. Ten per cent solutions of sodium nitroprussid in 1, 2, and 5 per cent sulphuric acid are still as good as when made, after six months in brown glass bottles. Since both the brown bottles and the sulphuric acid contribute to the stability of the solution, we now use either 2 or 10 per cent sodium nitroprussid in 2 per cent sulphuric acid, and keep the solution in a brown glass bottle. This amount of acid is of no consequence in using the qualitative acetone methods; in quantitative methods a corresponding excess of alkali may be used.

Recently it has come to our attention that Cavalli (1897) and Zucarri (1915),[1] both inorganic chemists, have reported that nitroprussid solutions are far more stable when kept in the dark and that small amounts of sulphuric acid added to the solution greatly increase this stability.

For the convenience of physicians, 10 per cent sulphuric acid made up with quantitative accuracy may be obtained from the Denver Fire Clay Company of Denver, Colorado.

*Received for publication, July 27, 1931.

†Hydrochloric acid decomposes nitroprussid quickly producing hydrocyanic acid. Nitric acid was not used because of its oxidizing action.

[1]Cited by J. N. Friend, A Textbook of Inorganic Chemistry, Vol. IX, Part II, p. 231.

A SPLIT SECOND TIMER*

By C. C. Guthrie, M.D., Ph.D., Pittsburgh, Pa.

FOR many laboratory purposes it is desirable to measure time in fractions of a second, as with stop watches. Aside from their high first cost, short life in student hands, and high cost of and unsatisfactory character of repairs of the usual Swiss works, they are convenient and satisfactory.

To eliminate some of these objections, an inexpensive, simple and accurate timing device has been designed to supplant watches for many such purposes.

It consists of a glass reservoir and graduated glass tube connected by a metal tap mounted on a rotating base, and a suitable quantity of cleaned and sifted sand. (See Fig. 1.) It is at least as accurate as the average stop watch.

To operate, the base *(8)* is rotated upon the friction support *(6)* until the reservoir *(1)* is down and the base is against a stop on the support and is nearly perpendicular. The tap *(3)* is opened and all sand in the graduated tube and connection is emptied into the reservoir. The tap is then closed and the base rotated to perpendicular when it comes in contact with the support stop and the reservoir is up. The timer is then ready for use, the tap being opened at the start and closed at the end of an observation. The time is read on the scale from the top of the sand column in the tube.

After the best coarseness of sand had been determined an article dealing with the most favorable coarseness of fertilizers for uniform distribution by machines came to notice.[1] Such materials having an average angle of repose of about 34° gave the best results.

The angle was determined by pouring the material into a conical pile upon a horizontal surface and measuring the angle between the surface and cone. This was done with the sand used in the timer and it measured within about a degree of that of the fertilizer.

*Received for publication, July 22, 1931.

[1]Mehring, A. L.: Industrial and Engineering Chemistry, 3: 34, 1931.

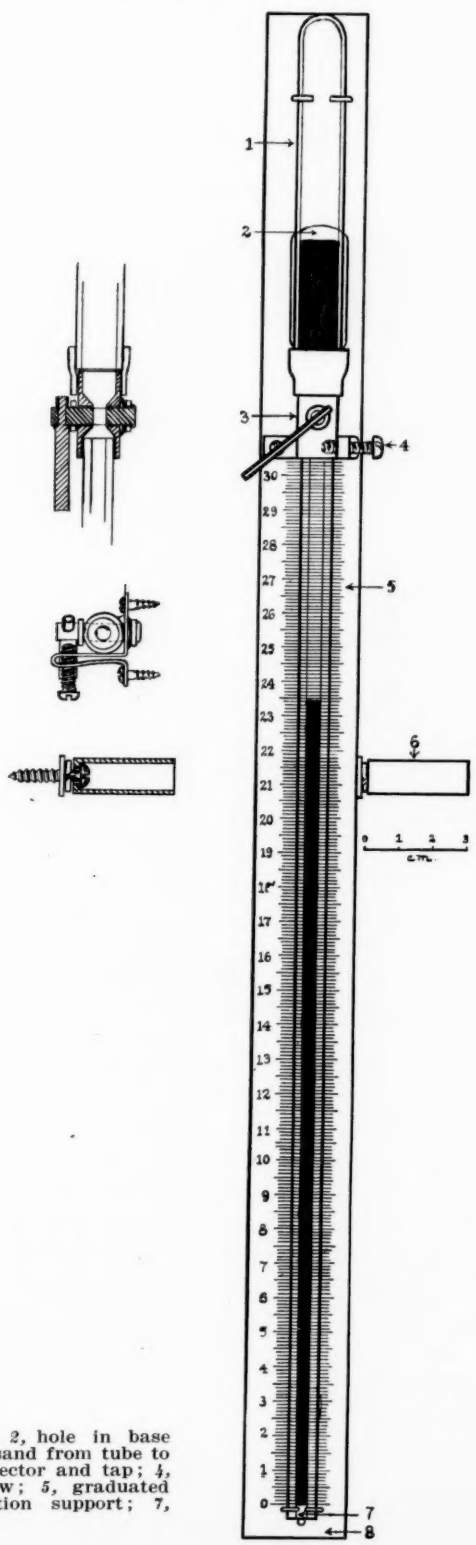


Fig. 1.—*1*, Reservoir; *2*, hole in base for observing return of sand from tube to reservoir; *3*, metal connector and tap; *4*, adjusting and stop screw; *5*, graduated tube; *6*, rotating friction support; *7*, plug; *8*, base.

A NEW DEVICE FOR HONING MICROTOME KNIVES ON GLASS*

By N. Graham Stabler, Philadelphia, Pa.

A DEVICE known as the *Schmid hand microtome knife sharpener*† has recently been placed on the market. The device makes use of a glass plate for honing, as does the Fanz automatic knife sharpener,[1] but is very simple in design and therefore very much less expensive. Compared to honing on stones, much time is saved, much less skill is required, and there are other advantages, inherent to all glass plate honing methods, which will be mentioned below.

This device consists (see Fig. 1) of a glass plate 14 by 14 inches, ½ inch

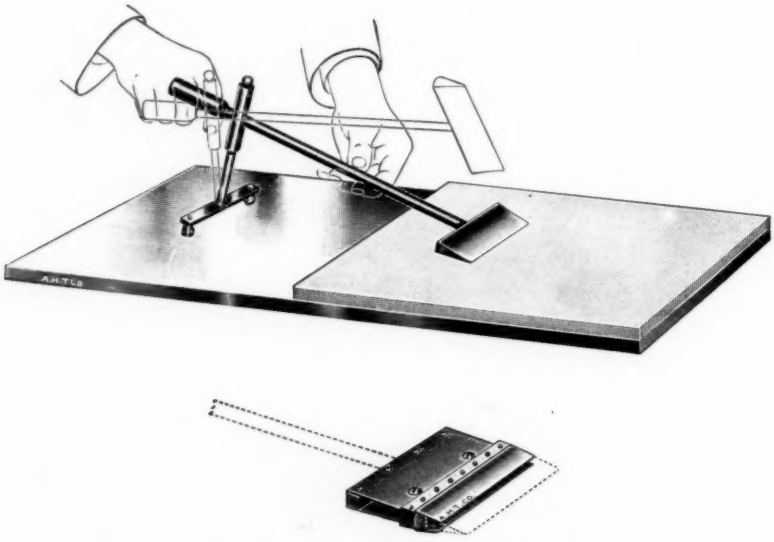


Fig. 1.—The microtome knife sharpener and clamp.

thick, ground on one surface, on which the honing is done; a glass base plate 14 by 28 by ½ inches; a phosphor-bronze clamp which will hold almost any microtome knife of detachable handle type; a stainless steel knife holder rod which screws into the clamp and has a convenient wooden handle on the other end; a stainless steel upright with two casters and with eight holes, any one of which can be used for the knife holder rod. The latter screws apart at the point where it fits the upright, and will rotate freely in its hole in the upright when in place. The outfit as sold includes in addition a set of abrasives and accessories. The knives furnished from now on by the maker of this device will have a threaded hole for the knife holder rod. With these knives the clamp is not needed.

*From the McManes Laboratory of Pathology of the University of Pennsylvania.
Received for publication, August 12, 1931.
†Sold by Arthur H. Thomas Co., Philadelphia.

The method of using is very simple, and while it requires reasonable care on the part of the user, it requires practically no skill. An inexpert girl has made a knife sharp the first time by following the written directions that come with it. For working a knife into shape the first time, and for rough honing, FFF emery flour is suspended in 5 per cent glycerin and poured on the honing plate, and the knife is worked rapidly in circles on it. For finish honing, white rouge is used, suspended in 5 per cent glycerin, and more care is exercised in honing, the knife being pushed edge first only. Anyone who so desires may use diamantine No. 1 for finer honing. It has recently been found, however, that an absolutely smooth edge is undesirable, at least for paraffin cutting, and it is believed for celloidin also. Experience indicates that there is an optimum degree of roughness of the edge for best cutting; and that white rouge seems to produce somewhere near this optimum degree. A knife honed on white rouge and stropped slightly cuts beautifully. It is the sharp uniform-grade abrasives that make glass plate honing speedier than even well-dressed stone hones.

The device seems to merit wide use because, first, its low cost, not a great deal more than a set of hones, puts it within the reach of every laboratory; second, because no user should have trouble developing what skill is necessary; third, because the honing surface will not wear out of shape as do stone hones; fourth, because any grade of abrasives desired may be used. Its only disadvantage, breakability of the glass, is not serious. The thick plate glass does not break easily, and is not very expensive to replace. This disadvantage is inherent in all glass plate honing methods.

REFERENCE

1. Fanz, J. I.: An Automatic Knife Sharpener and Methods for Grinding and Honing the Knife Satisfactorily, J. Lab. & Clin. Med. 14: 1194, 1929.

A DEVICE USED WITH MOUNTED INTESTINAL SPECIMENS TO SIMULATE SIGMOIDOSCOPIC VIEWS*

By Joseph Felsen, M.D., New York, N. Y.

PURPOSE.—Didactic. A simple optical arrangement to be used in connection with mounted intestinal specimens to simulate views obtained through a sigmoidoscope or proctoscope.

Principle.—A reflected image of a limited area of the specimen is viewed through a cardboard tube.

Apparatus.—(1) A well silvered plane surfaced mirror of high optical quality. (2) Gray cardboard tube of approximately the same length and diameter as a sigmoidoscope (12 by ¾ inches) or proctoscope (6 by 1 inches).

The mirror should be approximately six inches in its anteroposterior diameter and set at an angle of ten to fifteen degrees to the horizontal plane. The viewing tube, held by wire, cardboard or aluminum combination shield and support, is placed horizontally in such a position as to afford a view of the pathologic area to be demonstrated. The purpose of the shield, which is approximately the size of an ordinary filing card (3 by 5 inches) and which is slipped over the viewing tube between the eye and mirror, is to shut out confusing images of objects in the vicinity or of contiguous portions of the specimen. This may be modified by covering the mirror with a paper or cardboard sheet having a circular cut-out so as to limit the image to that part of the specimen being demonstrated. Where numerous specimens are available, mirrors may be cut into suitable lengths so as to form a continuous mirror shelf. When set up with neat cardboard folders (11 by 14 inches) containing brief summaries of the clinical

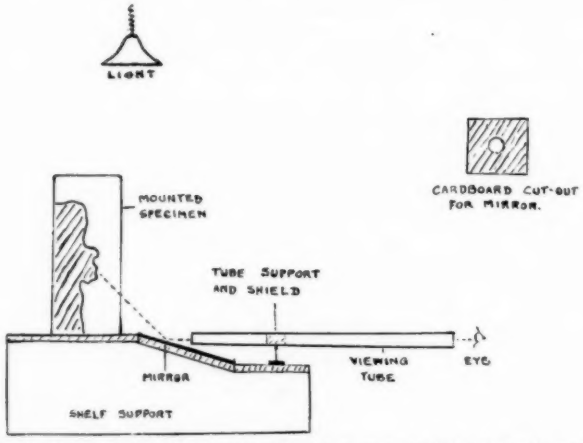


Fig. 1.—A pseudosigmoidoscopic device adapting mounted intestinal specimens for clinical study.

*Received for publication, August 13, 1931.

and pathologic aspects of the case as well as gross specimens and photomicrographs, the whole makes an impressive and very instructive exhibit. The source of illumination is from above, the ordinary pendant or gooseneck brackets being satisfactory. The image is upright, undistorted and of unusual clarity due to the

Fig. 2.—Photograph of mirror image seen through device as described. Photograph made with a 3x4 kodak, one minute exposure, 75 watt illumination, using a magnified doubly reflected image. (Photograph has been retouched.) (Felsen, J.: The Use of a Magnified, Doubly Reflected Image in Proctologic Demonstrations, J. A. M. A. 97: 23, 1931.)

presence of preserving fluid. There is no appreciable magnification where specimens are mounted in rectangular or square jars. The mirror-tube device, being below the general level of the specimen shelf, neither obstructs a direct view of the museum jars nor interferes with the illumination of specimens from above. This has a decided advantage over a tube aimed directly at the pathologic area.

A MICROSCOPIC ARRANGEMENT FOR READING MACROSCOPIC KAHN PRECIPITATION TESTS*

By David H. Flashman, M.D., New Kensington, Pa.

VARIOUS methods are in use for reading Kahn precipitation tests. One of the simplest is to use the inverted ocular eyepiece of a microscope. This method tends to result in scratching of the lens and does not give a high magnification. We have found the following method of using the compound microscope for this purpose to be such a decided advantage as to warrant its report.

The stage of the microscope is tilted at an angle of about 45 degrees. A simple wooden frame is constructed to support the small test tubes used in the test and to fit into the mechanical stage of the microscope. A mechanical stage is not necessary, but is very handy. Different types of mechanical stages will require different types of supports to fit them. We use a support composed of 2 side pieces of wood, each 4.8 by 1.2 by 0.4 cm., and of a baseboard 3.8 by 1.2 by 0.4 cm. The baseboard fits by friction into a space at the lower inner surface of our mechanical stage (Bausch and Lomb). The 2 side pieces of wood are nailed perpendicularly to the baseboard, so that the narrow sides rest semivertically on the stage and the wide sides are parallel to each other about 13 mm. apart, to support a test tube 12 mm. diameter and about 72 mm. long. Fig. 1 illustrates how the test tube fits into the frame, as both lie on the stage of the microscope. The side pieces should be shorter than the test tube to leave room to grasp the tube.

The upper surface of the liquid in the tube remains horizontal to the ground, but is slanted in relation to the long axis of the tube. The condenser lens of the microscope is best removed and the diaphragm beneath the stage is much narrowed. The magnification is about 12 times, using a binocular microscope with 5X oculars and a number 3 Zeiss objective. If the lower lens of a 16 mm. objective (Bausch and Lomb) is removed, a similar magnification can be obtained. One focuses on the surface of the slanted portion of the fluid, at the thinnest region. Under these conditions, with the light satisfactorily cut down and the mirror properly adjusted, the lower zone of the microscopic field will show a bright light while the upper one-half or more will show a dark-field illumination, in which the particles of antigen can be seen as luminous points against a dark background.

It is helpful to shake the tubes gently before inserting them in the frame, as the moving of the particles allows more particles to be seen. If the tube is scratchy, one can rotate the tube in the holder in order to look through that portion of the tube most free from scratches. Some experience with the apparatus is of course necessary, but we have found that beginners acquire skill in reading the tests more quickly than with the use of an eyepiece lens.

*From the Pathological Laboratory of the Citizen's General Hospital.
Received for publication, August 16, 1931.

The method permits greater sensitivity because the slightest degree of aggregation of the particles can be detected. The proportion of antigen and salt solution and the amount of antigen used naturally affect the dispersion of the particles as well as the sensitivity of the test. It is well to use that combination which gives with the antigen control and the negative sera a uniform, fine dispersion. With strongly positive sera, the clumps are large and few, and all degrees between this picture and that of the negative sera will be found. The method avoids mistakes due to foreign particles, such as red blood corpuscles, clumps of bacteria, etc., because the fine particles of antigen will still be readily seen if the serum is negative, but if positive there will be a uniform aggregation of the fine particles to larger ones. It is the aggregation of the finer particles that indicates most definitely a positive reaction, rather than the presence of larger particles. The results can be expressed as plus-minus, one-plus, two-plus, three-plus, and four-plus, depending on the degree of reaction.

With this method, the results are simpler by using only one amount of

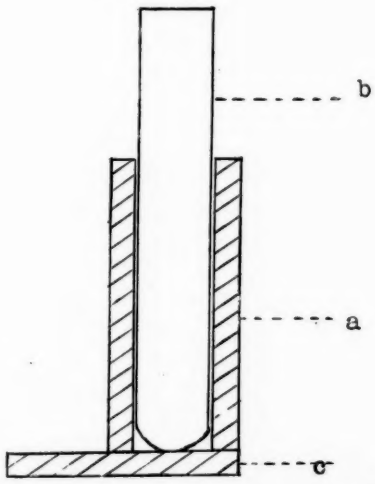


Fig. 1.—Support for test tube. (*a*) Side-piece to support test tube (*b*); (*c*) baseboard that fits into groove of mechanical stage. (Slightly reduced from natural size.)

antigen, instead of three as in the regular Kahn test, and the more sensitive quantity should be used. With the Kahn antigen sold by Parke, Davis & Co., we have found a 1 to 1.5 dilution of the antigen better than the 1 to 1 dilution recommended by the company. Also the use of 0.005 c.c. of this antigen dilution gave better results than the use of larger quantities. It is well also to use two different preparations of antigen, each adjusted to the most sensitive combination, as some serums react better with one antigen than with another. In this case the unknown sera tested with a given antigen must be compared with known negative sera tested with the same antigen.

Since the method promotes greater sensitivity by detecting the slightest degrees of reaction, more care must be used in the interpretation of these slight reactions. Inaccurate pipetting, unclean glassware, and other such sources of error would naturally be more apt to produce false slight reactions the more sensitive the method. Also the presence of other diseases would act similarly.

However, for treated cases of syphilis and as a supplement to clinical evidence, the slight reactions are helpful. One must bear in mind, however, that the less the degree of reaction, the lower the probability of the reaction indicating the presence of syphilis.

SUMMARY

A simple method is described for adapting the compound microscope for the reading of macroscopic Kahn precipitation tests. The stage of the microscope is tilted to an angle of about 45 degrees and one looks through the slanted portion of the fluid in the test tube, with the illumination cut down. A dark-field illumination of the particles of antigen is thus produced, giving a sensitive and easy method for reading the tests.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

BILIRUBINURIA: Methylene Blue Test for, Siede, J., and Zink, K. Deutsche med. Wchnschr. **57:** 1744, 1931.

To 5 c.c. of urine add, drop by drop, a 0.2 per cent aqueous solution of methylene blue. A blue color on the addition of the first drop indicates a negative reaction.

In the presence of bilirubin a green color appears. The number of drops of methylene blue required to change the blue color to green indicates the amount of bilirubin present (2 drops = 0.1 mg. of bilirubin).

The following scale is suggested:

More than 10 drops: strongly positive.

2 to 10 drops: weakly positive.

B. TUBERCULOSIS: Demonstration of Rare Tubercle Bacilli in Sputum, Pottenger, J. E. Am. Rev. Tuberc. **24:** 583, 1931.

The following method is advocated:

1. For collection of specimens and also for carrying out the technic use 8-ounce, wide-mouthed, graduated bottles, which are selected to fit the shaker. They are graduated for every 10 c.c. through paraffine, with hydrofluoric acid. No. 16 cork stoppers, to fit the bottles, must be used once only.

2. Siphons, 3 - 4 mm. lumen, for removing subnatant liquid.

3. Capillary pipettes with rubber bulb, for removing hydrocarbon layer in making preparation.

4. Open water-bath for digestion, kept at 55 to 58°.

5. Closed flat-top oven, containing water at 65° for drying films on slides, and for staining.

6. Mechanical shaker. The 4 bottle shaker manufactured by the International Equipment Co. has served well.

PROCEDURE FOR CARRYING OUT TECHNIC

1. Collect twenty-four-hour or three-day specimen.

2. Add equal parts of 0.5 per cent NaOH. Shake by machine for five to ten minutes.

3. Digest in water-bath 55° for thirty minutes to one hour.

4. Pipette off and discard any insoluble residue which has settled at the bottom of the bottle.

5. Add 1 to 2 c.c. hydrocarbon (xylol, ligroin, benzene, or gasoline) fill with distilled water to about 200 c.c. Shake ten minutes.

6. Allow hydrocarbon to collect at top of bottle (ten to twenty minutes).

7. If viscosity of specimen is too high, remove subnatant liquid by use of sterile siphon, fill to 200 c.c. again with distilled water; shake five minutes.

8. When viscosity is practically that of water, the supernatant hydrocarbon layer is drawn up in a sterile pipette which is pinched by inserting cork, and allowed to remain in vertical position until subnatant liquid separates from the hydrocarbon layer (five to ten minutes). The liquid is discarded and preparations are made from the hydrocarbon layer which is put on the slide and dried, layer upon layer, to desired thickness.

9. Wash preparation in ether to remove traces of hydrocarbon and fat.

10. Stain preparation as usual. In decolorizing with alcohol, do not allow more than twenty seconds' exposure, as bacilli decolorize rapidly. Complete decolorizing with 5 to 10 per cent sodium sulphite if necessary. Counterstain with 1 per cent aqueous picric acid.

PNEUMONIA: Immune Transfusion In Lobar, Barach, I. L., and Soroka, M. Am. J. M. Sc. **182: 81, 1931.**

Of 8 patients who were treated by immune transfusion, 6 were due to pneumococcus Type III and 2 to pneumococcus Type II. Four of the 6 patients of Type III cases had a bacteriemia and all died. The 2 patients of Type III cases with sterile blood culture survived. Of the 2 patients of Type II pneumonia with positive blood culture, 1 died and 1 survived.

The immediate effect of the transfusion on the clinical condition of the patient was negligible. No lessening of toxemia, prostration, cyanosis, or dyspnea was observed. In four fatal cases, the blood culture was not rendered sterile, nor was the spread of pulmonary consolidation checked. The determination of protective substance in the patient's blood before and after transfusion of immune blood showed an absence of the introduced protective substance in all the 5 fatal cases. Of the 3 who survived, 1 showed no protective substance in his serum until two days following the last transfusion, which therefore could not be ascribed to the introduction of immune blood. In 2 cases of pneumococcus Type III infection with sterile blood culture, the patient's serum contained protective substance after immune transfusion although not before. Their clinical course, however, was so long drawn out that it would not be possible to ascribe recovery to the protective substances introduced by transfusions.

Eight patients with pneumococcus lobar pneumonia, 6 due to Type III and 2 due to pneumococcus Type II, were treated by transfusion of 1000 to 1600 c.c. of blood from donors immunized over long periods of time.

No favorable effect was observed on the clinical condition of the patient as a result of any of the transfusions. The introduction of protective substance present in the donor's blood could not be demonstrated in the patient's blood in the 5 fatal cases and appeared to have no influence on the course of the disease in the 3 recovered patients.

TUBERCULOSIS: Direct Culture In Tuberculous Effusions, Bezancon, F., and Buc, E. Presse med. **39: 1493, 1931.**

The authors have had success in the culture of tubercle bacilli using the following medium: Peptone 2 gm.; glycerin 5 gm.; water 100 c.c.; neutralizing with 1 gm. of monobasic potassium or sodium phosphate. The medium was tubed (2 to 3 c.c. per tube) and sterilized at from 110 to 115 C. Pleural fluid was inoculated in quantity almost equal to the tubed medium and the two liquids were mixed. Growth in the liquid usually appeared the third or fourth week in the form of cotton filaments, sometimes at the bottom of the tube as small opaque white granules; these increased in volume and assumed an arborescent aspect. The growth was more vigorous close to the surface of the liquid, there assuming cottony colonies. The bacilli were acid-resistant, often highly granular, sometimes fragmentary, and virulent for the guinea pig.

In old thick, puriform, pleural effusions the microscopic examination was sufficient and animal inoculation was usually effective. For direct culture it was found necessary to add to the peptone glycerin medium 3 per cent gelose and one-third volume of blood serum. In making cultures of old puriform exudates, as in serious exudates, inoculations were made exclusively from effusions in the course of artificial pneumothorax.

NEUROSYPHILIS: Malarial Therapy of Other Than Uncomplicated Dementia Paralytica, Wile, U. J., and Davenport, K. M. J. A. M. A. **97: 1579, 1931.**

The authors thus summarize their experience since 1927:

1. Malarial therapy was used in the treatment of tabes, of dementia paralytica with tabes, and of diffuse neurosyphilis, including cases complicating the secondary stage.

2. In a large percentage of cases, immediate improvement was noted. Thus, 53 per cent of the tabetic group showed immediate improvment, and later observation increased this group to 67 per cent. In the greater number of these, improvement amounted to complete symptomatic remission. One patient showing immediate improvement relapsed later.

3. In patients with dementia paralytica with tabes, 40 per cent showed immediate symptomatic improvement; later observation increased this figure to 67 per cent. Thirteen

per cent of the cases were arrested; 13 per cent were made worse, and 7 per cent of the patients died after leaving the hospital.

4. The immediate results were most striking in the diffuse central nervous system group. In the group complicating secondary syphilis, all eight were immediately improved, seven later remained asymptomatic, and one subsequently relapsed. In this case, recommended therapy was not carried out.

5. Twenty-four cases of later occurring diffuse neurosyphilis showed striking immediate improvement in all but one case. The later follow up of this group showed improvement in 84 per cent, no change in 10 per cent, and a change for the worse in 6 per cent.

6. Ultimate gain in weight was an almost uniform feature, even in patients who did not otherwise improve.

7. Following treatment, many colloidal gold curves became negative, reversed, or became more or less intense without paralleling clinical results.

8. Reversal of the serologic reaction or diminution of its positivity occurred more often in the spinal fluid than in the blood in the group studied.

9. Decrease in cell counts and organic solids was almost invariably noted.

10. From the foregoing conclusions, it is apparent that malarial treatment is a definitely beneficial addition to the armamantarium of neurosyphilitic therapy.

B. TUBERCULOSIS: A Potato-Egg Medium for Isolation, Woolley, J. S., and Peter, F. G. Am. Rev. Tuberc. 24: 596, 1931.

Preparation of Medium.—

All glassware is sterilized in the autoclave; also egg beater, potato masher, etc. Eggs should be soaked in alcohol (70 per cent). The culture tubes, 6 × ¾ inches, are stoppered with a No. 8 long cork stopper and heated in a hot-air oven at 275° F. for one and one-half hours. The use of the cork stopper helps to retain the moisture during sterilization and later. It also facilitates the seeding of the tubes as the stoppers can be easily withdrawn.

Autoclave medium-sized unpeeled potatoes for thirty minutes at 15 pounds' pressure. Peel, and after thorough mashing, suspend in 15 per cent glycerol water, in the proportion of 500 gm. of original potato to 500 c.c. of 15 per cent glycerin. Heat in a boiling water-bath for thirty minutes, stirring occasionally. Strain through two layers of surgical gauze by compression into a flask and then boil filtrate for about five minutes. Cool and add one part of filtrate to two parts of well-beaten whole fresh egg. Add sufficient 1 per cent crystal violet to make a concentration of 1 to 30,000. Stir thoroughly, to distribute the dye evenly throughout the mixture. Pour the mixture through one layer of sterile gauze into a tubing funnel and add approximately 7 or 8 c.c. of medium to each tube. Sterilize in the inspissator at the proper slant for one-half hour at 85° C. on the first day, and at 75° on the second day. Bring inspissator up to required temperature before introducing tubes. By omitting the crystal violet an excellent medium is obtained for growing stock cultures of tubercle bacilli. Incubate the tubes to test for sterility. Tubes must be stored, seeded, and incubated in an upright position to keep the surface of the medium dry.

The sputum before isolation was treated as originally suggested by Corper:

One cubic centimeter of sputum is beaten into a homogeneous pulp and introduced into a sterile 15 c.c. centrifuge tube with 1 c.c. of "6 per cent sulphuric acid" (prepared by adding 17 c.c. of sulphuric acid, specific gravity 1.84, to distilled water of 500 c.c. final volume). After thorough mixing the tube is stoppered with a sterile cork and incubated at 37° C. for thirty minutes. During this period it is occasionally shaken. The contents are then diluted with about 10 c.c. of sterile physiologic sodium-chloride solution, well mixed and centrifugated. The supernatant fluid is decanted, and the residue cultured or inoculated without further treatment.

MENINGITIS: The Diagnostic Value of Smears from Purpuric Lesions in Meningococcus Bacteriemia, McLean, S., and Caffey, J. Am. J. Dis. Child. 42: 1053, 1931.

The following procedure was found to be of early diagnostic value in endemic purpuric meningococcus infections.

The technic of examining purpuric skin lesions for meningococci is simple. The skin surface over the cutaneous lesion is cleansed with alcohol and permitted to dry, and then a small stab wound is made with a Hagedorn needle. Uusually the largest lesions are selected for puncture. The examination, however, was satisfactorily completed several times with small petechiae. After the skin puncture, the extravasated blood and tissue juices from the lesion are smeared onto a slide or cover glass. They are then dried in the air and stained by Gram's method. Microscopic examinations are then made with the high power oil immersion lens. The material on the preparation varies considerably in its content of blood cells and polymorphonuclear leucocytes. Usually a surprisingly large number of the latter cells are present. Search is made until typical intracellular organisms are identified. In negative cases search was continued for at least one hour before a negative result was tabulated.

The results are positive only when the gram-negative diplococcus has been seen within polymorphonuclear leucocytes. Typical looking organisms were frequently present extracellularly as well as within the cells.

POLIOMYELITIS: The Spinal Fluid Cytology, Thelander, H. E., Shaw, E. B., and Lemper, M. A. Am. J. Dis. Child. 42: 1117, 1931.

In a series of 122 cases of poliomyelitis, the cell count of the spinal fluid varied from 10 to 700, with the greatest number of cases between 50 and 200 and about an equal number below 50 and between 200 and 300, dropping off rapidly over 300, but a few scattered cases occurring up to 700. A high percentage of cases of bulbar involvement may account for the large group with a low cell count.

About one-half of the cases had a polymorphonuclear percentage over 50, the peak of the curve being in the group from 50 to 75 per cent. This finding is not in accordance with most authors and may be accounted for by variations in different epidemics and by the technic of staining and studying the cells.

The percentage of polymorphonuclears during the acute phase of the disease was independent of the day of the disease. The presence of a high percentage of polymorphonuclears is dependent, therefore, probably on some factor other than the stage of the disease.

The blood count quite consistently showed a slight leucocytosis with a relatively high polymorphonuclear count.

ARTHRITIS DEFORMANS: Role of Streptococcus in. An Improved Cultural Method, Gray, J. W. and Gowen, C. H. Am. J. M. Sc. 182: 682, 1931.

The following modification of Cecil's technic is said to show growth in one to four days.

The patient's arm is prepared by two coats of iodin, washed off with alcohol and 20 c.c. of blood is drawn from a vein in the arm, and placed in two sterile dry test tubes (10 c.c. in each). These are placed in the ice box over night. The serum is removed, clot broken up, and the pieces of clot placed in each of two 100 c.c. bottles containing 50 c.c. of media. The media is prepared as follows: Fresh beef heart is freed from fat and fibers, ground finely in a meat chopper and infused at ice box temperature over night, using 500 gm. ground meat and 500 c.c. of tap water. The next morning the infusion is warmed from 20° to 25° C. and squeezed through a flannel bag. The filtrate is then boiled slowly for one hour and filtered through paper. It is then made up to volume, 1.5 per cent peptone (Witte), 0.5 per cent chloride of sodium, 1 per cent dextrose, and 1 per cent gelatin added. This is then placed in the Arnold sterilizer for twenty to twenty-five minutes to dissolve the ingredients. It is then titrated to PH 8 and laced in the Arnold sterilizer for one hour. It is filtered through paper and retitrated. If the PH has dropped below 7.8 it should be retitrated to that figure. It should not be below a PH of 7.8 before placing in bottles. The bottles are prepared beforehand by placing about a teaspoonful of calcium carbonate (c.p., powdered) in each of them, plugging with cotton, or cheesecloth, and cotton and sterilizing in the dry sterilizer for one hour. In these sterile bottles 50 c.c. of medium are placed and sterilized in the Arnold sterilizer for thirty minutes on three successive days.

At the end of three days it is titrated and if the PH is 7.6 to 7.8 it is satisfactory. It usually shows a PH of 7.7 to 7.8. If the PH is correct, the medium is placed in the incubator for several days and if sterile is then ready for use.

The authors thus summarize their investigation:

1. Our results confirm recent investigation that arthritis deformans is due to an Alpha type streptococcal infection of the joints.

2. Climate and fatigue are important predisposing factors.

3. The Alpha type or Alpha prime streptococcus causing arthritis deformans produces slight hemolysis in primary cultures.

4. The blood or joint fluid was positive for this streptococcus in 62 per cent of 71 arthritis deformans cases.

5. An improved cultural method for the quick growth of this organism from the blood and joint fluid is described.

6. The clinical picture and pathology of arthritis deformans are typical of an infectious process.

7. Agglutination tests are of considerable value in diagnosis.

8. The importance of general and focal treatment should not be underestimated.

9. Specific vaccine therapy is the most efficient form of treatment because it has cured or improved patients who were becoming progressively worse following other forms of treatment.

10. Vaccine should be prepared from blood or joint cultures when possible, otherwise from cultures from foci or from stock specific strains.

11. Vaccine treatment preliminary to the removal of foci, particularly badly infected tonsils, might prevent undesirable joint reactions.

12. Vaccine for the cure of joint infection must be continued for a long period of time.

13. Intravenous vaccine promptly relieves symptoms and probably controls the joint infection more quickly than subcutaneous injection.

14. The dose of vaccine and interval between injections should be so regulated that reactions do not occur, particularly joint reactions.

TISSUE: Method for Examination of the Appendix, Steinberg, B. Arch. Path. **12**: 598, 1931.

The method below is useful in determination of a perforation and the localization of the lesion.

An ordinary 5 or 10 c.c. syringe is partly filled with a weak solution of eosin. A needle is attached to the syringe, and the needle is introduced into the lumen of the appendix through its proximal end. A hemostat is applied over the appendix and needle to keep the needle in place and to prevent the escape of the eosin. The heomostat is applied over that part of the appendix which shows the hemostat markings made by the surgeon. The piston of the syringe is gently pushed down so that the eosin solution runs into the appendiceal lumen. At the point of perforation, the eosin escapes through the wall and marks the point of the perforation. This method of filling the appendix was found preferable to the introduction of the fluid by gravity. The slight pressure exerted was not found to produce artificial perforations in gangrenous appendices. If a permanent record is desired of the perforation and its location, iodized poppy-seed oil 40 per cent may be introduced instead of eosin and a roentgenogram taken.

The following method is suggested for cutting and sectioning the appendix.

After the appendix is received from the operating room, it is placed in a 10 per cent formaldehyde solution for from six to twenty-four hours. The organ is then removed from the fixative and cut longitudinally with a long and flat-bladed knife. The cut is begun at the tip of the appendix with the heel of the knife and carried longitudinally through the approximate middle of the organ. The appendix is supported gently with the left hand, and the knife is carried in a single cut to avoid an irregular surface. At the completion of the section, two equal halves are obtained. Each half of the organ shows the lumen, its contents, and the wall.

For histologic sections one or both halves may be used. Any method of handling the tissue preparatory to embedding may be employed. However, Steinberg believes it preferable to use alcohol for dehydration and chloroform for clearing. Either celloidin or Warthin's celloidin sheet method or paraffin embedding may be selected. The paraffin method requires more skill and care in preventing wrinkles and tears. Sections 6 microns in width are made through various levels in which the appendical lumen persists. From five to ten sections will give a composite picture of the entire organ. The sections allow the determination of the width of the lumen and the relation of the opposing surfaces, and make it possible to study the whole length of the appendix in a single or two sections.

GLANDULAR FEVER: Protozoal Nature of The Experimental Disease, Bland, J. O. W. Brit. J. Exper. Path. **12:** 311, 1931.

The results of this study are thus summarized:

1. The rabbit diseases, previously called "experimental glandular fever," which followed the inoculation of blood from two cases of human glandular fever is caused by protozoa of the genus Toxoplasma.

2. These protozoa closely resemble the Toxoplasma cuniculi and are immunologically identical with it. The disease they cause is indistinguishable from that produced by a strain of T. cuniculi.

3. The protozoa differ from ordinary T. cuniculi in their greater virulence for rabbits and in their power to infect monkeys in which animal they produce a disease very like human glandular fever.

4. No similar disease has been produced in rabbits of the same stock by control inoculations with normal rabbit or normal human blood or with blood from febrile people.

5. The evidence suggests that human glandular fever may be caused by the protozoa described, but this requires confirmation.

DAKIN'S SOLUTION Simple Test for Available Chlorine Strength, Etc., Ullrich, A. H. Am. J. Pub. Health **21:** 1257, 1931.

Apparatus.—

Pipette graduated to deliver 5 c.c. Dakin's or equivalent chlorine solution. (Note: Our test is based on the use of a 5 c.c. sample of the solution to be tested.)

Pipette with two graduations, the first to deliver 6.43 c.c. N/10 As_2O_3, the other to deliver 7.86 c.c. N/10 As_2O_3.

Two 2-ounce ground glass stoppered bottles to be marked No. 1 and No. 2 respectively for convenience.

1 c.c. medicine dropper for orthotolidin.

Reagents.—

Standardized N/10 As_2O_3 solution.

Standard orthotolidin solution.

Procedure.—

To each of the 2-ounce bottles add approximately 20 c.c. of distilled water and then exactly 5 c.c. of the solution to be tested.

To bottle No. 1 add standard arsenious acid solution as measured by the lower mark on the graduated pipette or 6.43 c.c.

To bottle No. 2 add standard arsenious acid solution as measured by the upper mark on the graduated pipette or 7.86 c.c.

Shake contents of both bottles.

Add 1 c.c. orthotolidin solution to each bottle.

Results.—

No color in either bottle, solution tested weaker than the accepted Dakin's solution.

Yellow to orange color in bottle No. 1 but no color in bottle No. 2, solution tested has correct strength for accepted Dakin's Solution.

Color in both bottles, solution tested is stronger than accepted Dakin's solution.

SPINAL FLUID: A New Test, Gruskin, B. Am. J. Clin. Path. **1:** 441, 1931.

Ten Wassermann tubes are set up in a rack. Into Tube 1 there is pipetted 0.3 c.c. of spinal fluid, into Tube 2 there is pipetted 0.23 c.c. of spinal fluid. To Tube 1 there is added

0.7 c.c. of physiologic saline, and to Tube 2 is added 0.77 c.c. of saline, bringing the volume in each tube up to 1.0 c.c. Into each of the other eight tubes 0.5 c.c. of saline is added. The fluid in Tube 1 is well mixed, by gently shaking or by pipetting, and 0.5 c.c. is transferred to Tube 3, mixing well and transferring 0.5 c.c. to Tube 5, and so on in alternate tubes until the ninth tube is reached, from this tube 0.5 c.c. is discarded. Then beginning with Tube 2, the fluid is well mixed and transferred similarly in series to Tubes 4, 6, 8, 10, discarding the 0.5 c.c. from Tube 10.

To each tube is then added 1.0 c.c. of starch-iodine solution. The tubes are carefully agitated until the color is uniform in each tube, and the color of each tube is read immediately. The final readings are taken one-half hour later.

The starch-iodine solution is made as follows:

One part aqueous iodine (0.1 gram in 1000 c.c. distilled water).

One part starch solution (0.75 gram in 100 c.c. of saline).

One part physiologic saline (8.5 grams NaCl in 1000 c.c. of distilled water).

One-tenth gram of fine iodine crystals will dissolve in the water in from three to four weeks. The water must be absolutely free from organic matter so that the solution of iodine will not be weakened by any reaction or standing. Soluble starch is used for the starch solution and is heated only until a clear solution is obtained. The solution need not be boiled and should not be used when it has become cloudy on standing. The glassware for the test should be chemically clean and dried by sterilizing in the gas oven, thus keeping it free from foreign matter, such as lint, dust and the like.

The readings are made in symbols as follows: decolorized tube, O; light blue color, L; standard blue of starch-iodine solution, B. Enclosing the letter in parentheses indicates a lesser value, thus, (L) indicates a very pale blue color and (B) a slightly affected standard blue color.

In normal fluid two tubes are slightly affected, and one tube is sometimes decolorized.

In tabes, from four to five tubes are affected and two of these are decolorized.

In paresis, from five to six tubes are affected and three of these are decolorized.

In meningitis, from seven to nine tubes may be affected, according to the severity of the case and from five to six of these are decolorized.

PHOTOGRAPHY: X-ray Ink, Raison, T. W. Radiography **7:** 13, 1931.

The following formula, devised for marking x-ray films, conceivably might be used for marking microphotographs.

Water	20 c.c.
Sodium iodide	11 gm.
Barium sulphate	40 gm.
Mucilage of Acacia	2 gm.
Chloroform	1 c.c.

The sodium iodine content can be varied from the amount stated in the formula; more iodide gives greater x-ray absorption, although poorer writing and drying qualities, while the reverse is true for lesser quantities of iodide.

Since the barium sulphate tends to settle on standing, the ink should be well shaken before using. A *ball pointed* pen is recommended for use with this ink, and the writing should be done rather slowly in order to produce a broad, heavy line.

POLIOMYELITIS: Results of Treatment in One Hundred and Four Cases, Shaw, E. B., Thelander, H. E., and Lemper, M. A. J. A. M. A. **97:** 1620, 1931.

The study is thus summarized:

One hundred and four patients with poliomyelitis were admitted to Children's Hospital in the period from July 1 to Dec. 31, 1930. Specific therapy was attempted in 92 cases.

Of 53 patients treated before the onset of paralysis, 28 showed no paralysis at any time, 15 showed transient weakness which had entirely disappeared before dismissal, 9 showed persistent paralysis and one died.

The average age of the unparalyzed patients was nine and one-half years, of the transiently paralyzed, 10 years, and of those with definite paralysis, 17 years. This is at least significant to the hypothesis that better results are obtained in the lower age group.

The average spinal fluid cell count was 146 in the unparalyzed group, 119 in the transiently paralyzed group, 197 in the persistently paralyzed group, and 270 in the single fatality. These averages were made up from widely varying individual cases and, we believe, are without significance.

Serum was applied, on the average, 2.7 days after onset of symptoms in the unparalyzed groups, 3.6 days in those with transient paralysis, and 3.4 days in those with persistent paralysis.

The average amount of serum used in the group treated preparalytically was, respectively, 120, 151, 209, and 375 c.c.

In the group treated in the acute stage after the appearance of demonstrable weakness, of 39 patients, 9 showed transient weakness; 23 had persistent paralysis, and 7 died.

The average age in those with transient weakness was 6.8 years, 11.7 years in those with persistent paralysis, and 19.8 years in the fatal cases, again showing the higher average age in those with serious outcome.

Average cell counts were 67 in the transiently paralyzed, 194 in those with persistent paralysis and 199 in the fatal cases.

Treatment was instituted on the average of 3.7 days after onset of symptoms in the transiently paralyzed, 4.2 days in the cases of paralysis and 6.3 days in the fatal cases, coinciding with the general idea of the importance of early treatment.

The transiently paralyzed patients received an average of 84 c.c. of serum or plasma, the permanently paralyzed 156 c.c. and in the fatal cases, 156 c.c. was given.

Of the 53 patients treated before the onset of paralysis, 83.4 per cent showed no permanent paralysis; 16.9 per cent showed definite persistent paralysis, and 1.9 per cent died. Of the thirty-nine patients treated after the onset of paralysis while the disease was still acute, 23.08 per cent showed no end paralysis, 59 per cent showed definite paralysis and 18 per cent died. It is unfair to attempt to compare the results in these two groups of cases. While the first group included at least a few benign cases, the second group included many extremely virulent cases referred to the hospital because of their fulminant course.

MULTIPLE SCLEROSIS: Study of the Etiology of. Weil, A. J. J. A. M. A. **97:** 1587, 1931.

Having first learned their methods by working with Chevassut and Purves-Stewart the author repeated their work on the etiology of multiple sclerosis with the following results:

1. Repetition of the experiments of Chevassut and Purves-Stewart failed to produce convincing evidence that, in multiple sclerosis, cultures from spinal fluids yield a filtrable virus and that this virus is responsible for the production of the disease.

2. The fact that spheres and colonies of spheres may more readily be seen in agar cultures of spinal fluids that have given a positive globulin reaction suggests the precipitation of colloidal protein (or lipoid) particles, which become visible in the dark-field.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Klinische Laboratoriumstechnik*

THIS third volume consists mostly of functional tests of various organs such as the liver, kidneys, pancreas, stomach, intestines, and lungs; the gall bladder and extrahepatic bile ducts; the heart, blood vessels, and the circulatory system as a whole; and the organs of internal secretion such as the thyroid, hypophysis, adrenals, gonads and pancreas. There are also chapters on capillaroscopy, vital staining, in vitro cultivation of tissues, serologic diagnosis of cancer, and methods of testing the physiological effects of athletic training. A large number of tests are given under each heading and for each test not only are the apparatus used and the methods employed fully described but a critical evaluation is made of each test and a full bibliography added.

Die Regulierung der Atmung†

THIS monograph is a companion volume to the author's "Regulierung des Blutkreislaufes" (Thieme, 1930). It consists of a thorough discussion of respiratory phenomena. The action of such chemical agents as carbon dioxide, oxygen, acetylcholine, pituitrin, epinephrine, etc. are discussed and their effects correlated with circulatory, nervous, and mechanical factors. The author also offers new evidence concerning the Hering-Breuer reflex, in which he lays much stress upon the part played by the diaphram. A bibliography of over 400 titles is affixed.

Die speziellen Blutkrankheiten im Lichte der qualitativen Blutlehre‡

IN this second volume the author considers in detail the blood pictures encountered in various pathological conditions, grouping them according to the type of blood reaction produced.

He devotes 90 pages to agranulocytic reactions, 90 to lymphocytic reactions, 150 to lymphatic-leukemic reactions and the rest of the book to the lesser reactions. His method of differential analysis of blood cells resembles the better known method of von Schilling but differs from it in many details.

*Klinische Laboratoriumstechnik (Clinical Laboratory Methods). By Brugsch and Schittenhelm. Vol. III. Published by Urban and Schwarzenberg, Berlin and Vienna. 1928.

†Die Regulierung der Atmung (The Regulation of Breathing). By W. R. Hess. Georg Thieme, Leipzig. 1931. 137 pages.

‡Die speziellen Blutkrankheiten im Lichte der qualitativen Blutlehre. (Special blood diseases in the light of qualitative blood examinations.) By Joseph Arneth. H. Stenderhoff, Munster. 1930.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

The Journal of Laboratory and Clinical Medicine

VOL. XVII ST. LOUIS, MO., JANUARY, 1932 NO. 4

Editor: WARREN T. VAUGHAN, M.D.
Richmond, Va.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - - - - - CINCINNATI
PAUL G. WOOLLEY, M.D. - - - - - - LOS ANGELES
J. J. R. MACLEOD, M.B. - - - - - ABERDEEN, SCOTLAND
W. C. MACCARTY, M.D. - - - - - - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - - - - - - COLORADO SPRINGS
VICTOR C. MYERS, PH.D. - - - - - - - CLEVELAND
RUSSELL L. HADEN, M.D. - - - - - - - CLEVELAND
JOHN A. KOLMER, M.D. - - - - - - PHILADELPHIA
ROBERT A. KILDUFFE, M.D. - - - ATLANTIC CITY, N. J.
GEORGE HERRMANN, M.D. - - - - - - - GALVESTON
T. B. MAGATH, M.D. - - - - - - ROCHESTER, MINN.
DEAN LEWIS, M.D. - - - - - - - - BALTIMORE
M. H. SOULE, SC.D. - - - - - - ANN ARBOR, MICH.

Contents of this Journal Copyright, 1932, by The C. V. Mosby Company—All Rights Reserved

Entered at the Post Office at St. Louis, Mo., as Second-Class Matter. Additional entry as Second-Class Matter at Fulton, Mo., Pending.

EDITORIAL

Experimental Studies of Dengue

TIME was when diseases could be labeled geographically, as it were, and spoken of as "tropical," "Oriental" and the like with some confidence that necessity for any familiarity with their characteristics rested mainly upon physicians practicing in appropriate localities.

The changes brought by modern methods of rapid transportation, however, have almost erased the geographic boundaries of disease and to that extent have complicated and extended the scope of the diagnostic problems in which the practice of medicine abounds.

For these reasons, as well as for the interest which attaches to the clarification of any puzzling problem in medicine, the recent comprehensive report upon studies in dengue by the United States Army Medical Department Research Board constitutes a contribution of distinct importance.[1]

Dengue, or "breakbone" fever, is an acute disease characterized by sudden onset, initial erythema, headache, and pains in the trunk and limbs, especially

[1]Simmons, J. S., St. John, J. H., and Reynolds, F. H. K.: Experimental Studies of Dengue, Philippine J. Sc. 44: 1, 1931.

the joints, the pain and stiffness in which lead to a peculiarity in gait from which the term "dandy fever" arises.

The disease has long been the subject of experimental investigation.

That the etiologic agent of dengue is a filterable virus which can be demonstrated in the circulating blood and that the disease may be transmitted by the mosquito (Aedes aegypti) was first shown by Ashburn and Craig in 1907, their results being corroborated by the later work of Siler, Hall, and Hitchens in 1922.

The investigations thus begun have been continued and extended by the authors of the present report whose studies were planned to consider:

a. The epidemiology of dengue.

b. The nature of the virus.

c. The possibility of transmitting the disease by insects other than A. aegypti.

d. The possibility of transmission of dengue from infected to normal insects.

e. The search for a susceptible experimental animal.

f. Search for improved diagnostic methods.

g. Observations on the therapeutic value of immune sera.

h. Investigations of a prophylactic vaccine.

While all of these problems have not been solved, the investigation has definitely added to the present knowledge of dengue and will further the progress of future investigations not only of dengue but also of yellow fever, as the two diseases, though differing greatly in severity, as dengue is practically never fatal, nevertheless have many points of similarity.

1. The Epidemiology of Dengue.—The present studies indicate that, as far as the Philippine Islands are concerned, dengue occurs in seasonal waves reaching their peak sometime between April and November. There is a direct relation between the monthly rainfall, and hence the mosquito prevalence, and the prevalence of dengue. The suggestion is advanced that the dengue virus may be maintained in an endemic area by transfer through (a) susceptible native children, (b) incompletely immunized native adults, (c) susceptible newcomers from dengue-free localities, and possibly, (d) by direct transfer from infected to normal insect vectors.

2. The Transmission of Dengue by Aedes Aegypti.—The importance of this mosquito as a dengue vector is again substantiated by the present investigation which has added to the understanding of the mechanism involved.

It appears definitely demonstrated that the mosquitoes become infected only when they feed during the first forty-eight hours of the disease; that the disease may be transmitted by small lots of five or ten just as consistently as by large groups of one hundred to one hundred and fifty; and that infectivity persists for from sixty to seventy days, if not indefinitely at the temperatures prevailing in Manila (maximum, 36.7°C., mean, 26.3°C., and minimum, 17.4°C.).

3. The Transmission of Dengue by Insects Other Than A. Aegypti.—The investigations described in the report offer positive proof that not only A. aegypti but also A. albopictus transmit dengue and hence suggest that it is reasonable to suspect that still other members of the Aedes species may be concerned in the spread of this disease. Experiments with Culex quinquefasciatus indicate that

if this mosquito is able to transmit dengue to man, this probably occurs only as a result of mechanical transfer of blood during interrupted feeding. That is, a C. quinquefasciatus, interrupted in its feeding upon a dengue-infected individual, when it resumes feeding upon a noninfected individual, may mechanically transmit the virus. While such mechanical transmission of the disease has been proved experimentally, relatively large numbers of mosquitoes are necessary for such transmission, and it is probable that it occurs but rarely in nature.

Contamination of the skin by crushed A. aegypti or other insects in which the virus might exist does not appear to be a factor in the natural spread of dengue.

The conclusions of Siler, Hall, and Hitchens that the virus of dengue is not transmitted from infected female A. aegypti through egg to offspring are corroborated by the present commission, nor was it possible to infect mosquito larvae with the dengue virus, although it was not possible to be certain that the dengue virus added to the water was ingested by these larvae or that it remained alive long enough to cause infection.

As dengue virus escapes from the proboscis of infective female A. aegypti while they are feeding on blood, it was considered possible that a similar escape of the virus might occur during the ingestion of other foods or water and thus make it possible for normal mosquitoes feeding subsequently on the contaminated material to become infected. While this possibility was not proved experimentally, the evidence was suggestive and the contingency cannot be dismissed; particularly as in other experiments it was shown that the virus of dengue could be transferred by feeding normal A. aegypti on infected mosquitoes macerated and suspended in normal blood.

Under these circumstances not less than five infective mosquitoes per cubic centimeter of blood were required, a period of more than seven days being required before mosquitoes thus infected could transmit the disease.

4. The Nature of the Virus.—Attempts to demonstrate the dengue virus were entirely unsuccessful although attempted with various ways.

The results of cultivation experiments were entirely in accord with those of previous investigators in suggesting that the virus of dengue was incapable of multiplication in the culture media used (Noguchi's serum medium, Boeck and Drbohlov's ameba medium, Hibler's brain medium, M.N.N. medium, brain agar, Frances' cystine agar, dextrose infusion broth, and mosquito broth under aerobic and anerobic conditions at 37.5°C. and room temperature).

From available information it was evident that the dengue virus remains alive in its mosquito and animal hosts for much longer periods than in the dead tissues of these hosts, although the duration of life in the latter under favorable conditions may not be inconsiderable. The virus was not preserved in an infective state in dessicated infective mosquitoes stored at 18°C. for four days nor in frozen dried blood collected during the first day of fever.

The virus was again shown to be filterable during the course of the present experiments.

5. The Clinical Aspects of Dengue Fever.—Based upon experimental cases, the clinical aspects of the disease may thus be summarized:

The average incubation period was 5.66 days; duration of fever 4.8 days; with primary rash, 33.8 per cent; with secondary rash 69.5 per cent.

Additional symptoms commonly observed were leucopenia (100 per cent); postorbital pains and backache (90 per cent); altered sense of taste (65 per cent); pains in limbs (60 per cent); adenopathy (47 per cent); pains in the joints (43 per cent).

In 58 per cent of the cases (60) the onset was abrupt, usually with chill, while in 81 cases the disease was mild in 13.6 per cent, average in 70.4 per cent, and severe in 16 per cent.

From the diagnostic standpoint the leucopenia and other associated leucocytic signs were of the greatest diagnostic value, no significant changes being observed in the red cells or blood platelets.

Leucopenia was invariably encountered at some time during the infection, after its initial appearance was progressive, and often lasted for several days after the subsidence of the fever.

As a rule, the leucopenia began on the second day, reaching a low point in the fourth, fifth, or sixth day after onset, and gradually returned to normal on the third or fourth day of convalescence.

Differential studies showed the leucopenia to be due to a decrease in both the lymphocytes and mature neutrophilic granulocytes, the former first returning to the normal level during convalescence.

Coincident with the appearance of the leucopenia, there was an enormous increase in the number of immature granulocytes. This sharp "shift to the left" was a constant reaction and, together with the leucopenia, constituted the most reliable single diagnostic sign of dengue fever.

6. Dengue Immunity.—No evidence has yet been advanced to prove that all people of any race or group may be naturally immune to dengue. On the contrary, a large proportion of human beings are apparently naturally susceptible as is indicated by different epidemics involving from 75 to 100 per cent of inhabitants, particularly in places where the disease is first introduced.

That immunity is produced is shown by studies of adult natives living in endemic areas, the immunity in Filipinos being apparently well developed, although not necessarily invincible under experimental conditions.

The present studies suggest that the immunity following an attack of dengue fever in persons residing in endemic areas is probably completely protective in the majority of instances. There is some possibility, although this remains to be demonstrated, that the immunity produced by a single attack may be maintained by subsequent introduction of virus from infective mosquitoes.

Attempts, during the fever and following recovery, to demonstrate specific antibodies in the serum were not successful.

7. Prophylactic Vaccination.—While only failure followed vaccination of volunteers with filtrates from saline suspensions of macerated infective mosquitoes, saline suspensions of noninfective immune dried blood, and a vaccine prepared from infective A. aegypti, Simmons, Reynolds and St. John believe that an effective prophylactic vaccine against dengue may yet be developed by further investigation.

8. The Search for a Susceptible Experimental Animal.—Proof is offered that dengue can be transmitted to monkeys (M. fuscatus and M. philippinensis) by infected A. aegypti; that the virus multiplies in the blood without producing recognizable typical changes in the temperature or the leucocyte counts; that at some time between the third and ninth day the virus may be passed to other monkeys by blood inoculation or may be transferred through Aedes to other monkeys or man; and that in one monkey a single attack produced an immunity which lasted for several days.

As, therefore, monkeys are able to take part in the natural dissemination and maintenance of the virus in endemic tropical localities they are of considerable importance to any consideration of the epidemiology of dengue.

Attention is also called to the fact that negative conclusions should not be drawn concerning animal susceptibility unless: (a) the animals tested are known to have been protected throughout their lives from the bites of infected mosquitoes, and (b) unless they are tested daily from the fourth to the eighth or ninth day after the introduction of the virus by transfer of blood, either directly or indirectly through mosquito vectors to susceptible volunteers.

So closes a report representing a long and careful series of investigations no reference to which would be complete without mention of the volunteers whose willingness to become the subject of experiment made the investigation possible and in no small measure contributed to the measure of the success it attained.

—*R. A. K.*